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THE EFFECT OF NALTREXONE ON NICOTINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

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ABSTRACT

THE EFFECT OF NALTREXONE ON NICOTINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

By

Jonathan M. Adams

Nicotine is the central active ingredient in tobacco. The reinforcing effects of nicotine can be studied in animals through self-administration, conditioned place preference, and other approaches that enable researchers to assess nicotine cessation strategies. One strategy involves the use of opioid receptor antagonists. For instance, naloxone has been shown to reduce place preference for nicotine in rats, and other experimental opioid antagonists have also been shown to affect place preference for nicotine. The present study sought to extend these findings to the opioid antagonist naltrexone, which has long been FDA-approved for the treatment of opioid and alcohol addiction in humans. Using standard two-chamber shuttleboxes, we first sought to establish a place preference for nicotine in rats, and once this was achieved, we sought to block nicotine place preference with naltrexone. In the first experiment, subjects did not show a place preference for nicotine, but an alteration in the environmental stimuli used on the shuttleboxes led to a conditioned place preference for nicotine in the second experiment. In the third experiment, naltrexone did not block nicotine place preference. These results coincide with past findings that indicate a difficulty to establish a conditioned place preference for nicotine. This paper discusses these challenges and suggests other ways to evaluate a potential use for opioid receptor antagonists for treating nicotine addiction.

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This thesis follows the format prescribed by the in the *Publication Manual of the American Psychological Association, Sixth Edition.*

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INTRODUCTION

First- and second-hand tobacco exposure has been the subject of a barrage of public health warnings and ad campaigns over the past several decades due to links to cardiovascular disease, chronic obstructive pulmonary disease, and several varieties of cancer – most notably lung cancer, but also cancer of the mouth, pharynx, esophagus, kidneys, bladder, and other cancers (CDC, 2012). According to Benowitz (2010), an average of 435,000 Americans die of smoking-related ailments per year (accounting for one out of every five deaths), and the average lifelong tobacco smoker has a 50% chance of dying prematurely from a tobacco-related cause. In addition to the deleterious health effects of smoking, another potential issue with tobacco use is its potential for interaction with other drugs – especially with prescription medications. For instance, Porter, Heath, and Rosecrans (1994) found that nicotine can reduce the anxiolytic effects of the benzodiazepine diazepam, which means, for example, that a person suffering from anxiety may inadvertently negate the effects of his or her anxiety medication by smoking.

Seventy percent of smokers claim that they want to quit, but less than 5% do so successfully (Benowitz, 2010). Thus, a major dilemma is how to develop treatment strategies for smokers and other tobacco users to successfully quit using these harmful products. While a small proportion of tobacco users are able to quit smoking by simply ceasing use (quitting "cold turkey"), the one-year abstinence rate for smokers who quit on their own without help is only 6 percent (Livingston & Lynm, 2012). One of the major issues in the treatment of any substance use disorder is known as protracted withdrawal syndrome, or post-acute-withdrawal syndrome. Chronic drug use results in neuroadaptations involving a wide variety of neurotransmitters and brain circuits,

including those underlying motivation and decision making (Paolini & De Biasi, 2011; Zorrilla, Valdez, & Weiss, 2001). These adaptations result in widespread changes in neurotransmission, and the brain essentially establishes a new equilibrium in response to constant drug exposure. Withdrawal refers to the collection of somatic and affective symptoms that occur when this drug-specific equilibrium is disrupted, as in the case of drug cessation. Nicotine withdrawal symptoms can include concentration problems, irritability, anxiety, depression, and insomnia, and these symptoms can overwhelm the recently abstinent smoker and drive him or her to resume smoking in order to alleviate these unpleasant symptoms (Paolini & De Biasi, 2011).

In the case of post-acute-withdrawal syndrome, some symptoms may continue to persist for a much longer period of time than the typical acute withdrawal period, which can affect long-term abstinence success rates. Post-acute withdrawal syndrome is known to occur for several drugs of abuse, most notably alcohol, with which some withdrawal symptoms can be seen as long as 4 years following cessation of alcohol use (LeBon, Murphy, Staner et al., 2003). There is some evidence that post-acute-withdrawal symptoms may occur to some extent with nicotine as well, as evidenced by reports of nicotine craving 6 months following smoking cessation (Hughes, 1994).

One of the major avenues of treating substance abuse is through medication, or pharmacotherapy, which is often used as an adjunct to more traditional means of treatment, and has proven in recent years to be a very promising area for those who are seeking improved ways of treating substance abuse and dependence, either as a standalone treatment or in conjunction with other forms of treatment as part of a more holistic, comprehensive approach. Several different pharmacotherapies have been

developed for smoking cessation and are classified into three types: nicotine-replacement therapy, nicotinic-receptor agonists, and antidepressant drugs (Polosa & Benowitz, 2011).

Nicotine replacement therapy attempts to relieve the withdrawal symptoms of smoking cessation by providing an alternative source of tobacco-free nicotine via a skin patch, lozenge, nasal spray, chewing gum, or a variety of other delivery methods, many of which are available over-the-counter. The eventual goal of nicotine replacement therapy is for the user to follow a schedule to gradually wean him-or-herself off of the product so that they can be not only tobacco-free, but nicotine-free as well. Unfortunately, the 6-month smoking abstinence rate for over-the-counter nicotine replacement therapy is less than 10% (Lee & Tyndale, 2006). According to Basham and Luik (2012), some recent studies have indicated that nicotine replacement therapy may even be less effective than simply quitting tobacco use "cold turkey" without assistance.

However, some prefer an approach to tobacco cessation that does not involve the continued use of nicotine, which is where alternative pharmacotherapies come in. One such approach involves the use of antidepressant drugs. According to Hughes, Stead, Hartmann-Boyce, Cahill, and Lancaster (2014), there are three primary reasons why antidepressant drugs may be effective in aiding tobacco cessation: 1.) nicotine withdrawal may cause depressive symptoms that would be alleviated by antidepressant drugs; 2.) nicotine itself may serve as an antidepressant drug of sorts, thus antidepressant drugs may serve as an effective substitute in this capacity; and 3.) it is possible that some antidepressant drugs may affect the same neural pathways or receptors that underlie nicotine addiction (i.e.- they may inhibit monoamine oxidase or block nicotinic receptors). However, buproprion (Wellbutrin, Zyban), the most commonly prescribed

antidepressant drug for nicotine dependence, has shown to produce a 12-month tobacco abstinence rate of less than 25% (Lee & Tyndale, 2006).

Another nicotine-free approach involves nicotinic receptor agonists, which reduce the activation of the specific receptors that nicotine binds to in the brain, lessening the pharmacological effects produced by nicotine intake, as well as reducing cravings. In other words, when a tobacco user smokes a cigarette, he or she doesn't experience the same pleasurable effects as usual, thus rendering smoking less enjoyable. The first nicotinic receptor agonist to be FDA-approved for clinical use was the nicotinic $\alpha_4\beta_2$ partial agonist varenicline (Chantix). However, this strategy has not been shown to be overly successful either, as it has been shown to reduce tobacco use in less than 50% of smokers (Gonzales et al., 2006; Polosa & Benowitz, 2011).

Research has indicated that opioid receptors also may play a role in certain aspects of nicotine addiction (Jackson, Carroll, Negus, & Damaj, 2010; King & Meyer, 2000; Liu, et al., 2009; Rustkalis et al., 2005; Smith et al., 2005; Trigo, Martin-Garcia, Berrendero, Robledo, & Maldonado, 2010), which suggests that opioid receptor compounds may have utility for the treatment of nicotine addiction. In their comprehensive review of research involving the endogenous opioid system, Trigo and colleagues (2010) conclude that opioid receptors (primarily mu-opioid receptors, and to a lesser extent, delta-opioid receptors) are indeed critically involved in the rewarding properties of a variety of different drugs, including nicotine. Early proof of concept investigations will likely involve opioid compounds that have been FDA approved, and thus already screened through toxicology testing, for other purposes. The opioid receptor antagonist naltrexone has been FDA-approved as a treatment option for both opioid and

alcohol-use disorders, leading to a variety of studies that have examined the potential of this drug as a treatment for a variety of other substance use disorders (Modesto-Lowe & Van Kirk, 2002). As we will see in the following sections, results suggest that opioid receptor antagonism may be useful in the treatment of nicotine addiction, but naltrexone has yet to be FDA-approved as treatment option for anything but alcohol and opioids, so further research is warranted. Before exploring its relationship with nicotine, let us first examine naltrexone's method of action in the body and its application to substance abuse treatment in general.

Pharmacodynamics of Naltrexone

Naltrexone is one of three clinically available opioid antagonists, with the other two being naloxone (which is very short-lasting and used primarily for tests of opioid dependence or intervention in cases of opioid overdose) and nalmefene (which is used primarily in cases of opioid overdose to treat acute respiratory depression). Naltrexone is a nonselective opioid receptor antagonist, in that it binds with three of the primary subtypes of opioid receptors (Jayaram-Lindstrom, Konstenius, Eksborg et al., 2008; Mannelli, Peindl, Masand, & Patkar, 2007). According to Mannelli and colleagues (2007), naltrexone has the highest affinity for mu-opioid receptors (receptor binding Ki: 0.37 nM), but also has an affinity for kappa-opioid receptors (4.8 nM) and for delta-opioid receptors (9.4 nM).

Naltrexone and Opioids

Like the older opioid receptor antagonist naloxone, naltrexone administration precipitates withdrawal symptoms in opioid-dependent users by blocking the acute effects of opioid agonists. Whereas the shorter-acting naloxone is typically used to

counteract the effects of opioid overdose in current opioid users (McMenamin, 2012), the longer-acting naltrexone may be more suitable as a maintenance treatment for recently abstinent opioid-dependent users. Long-term naltrexone treatment not only blocks the intoxicating and rewarding effects of opioids in the case of relapse, but is also viewed by some as a safer and more socially acceptable option than traditional opioid agonist treatments (i.e.- methadone), as it provides no euphoric effects and is not addictive (Krupitsky et al., 2011; Sullivan et al., 2006).

Olmstead and Burns (2005) studied the effects of naltrexone administration on opioid withdrawal in rats, using conditioned place preference and conditioned place aversion paradigms. They found that co-administration of naltrexone in ultra-low-doses (5 ng/kg and 30 pg/kg) blocked the acute rewarding effects of oxycodone and morphine, respectively, as well as the anhedonic withdrawal symptoms associated with long-term administration of both drugs. However, in human subjects, Tompkins et al. (2010) found that co-administering ultra-low-dose naltrexone with oxycodone did not decrease abuse liability in experienced opioid abusers. According to Duggan and Lesley (2010), this may have something to do with medical compliance and route of administration. They studied the effects of a new anti-abuse delivery method that combines morphine and naltrexone in a single capsule. In the event that the capsule is tampered with (i.e. - crushed for purposes of intranasal or intravenous administration), naltrexone is rapidly released and absorbed, completely blocking the effects of morphine.

Sullivan et al. (2006) studied the effects of injectable depot naltrexone (another delivery method designed to reduce abuse liability and medical non-compliance) on clinical outcomes for heroin dependence in humans. They found that 384 mg of

naltrexone delivered via intramuscular injection blocked both the subjective and reinforcing effects of heroin for up to five weeks. After the second week, subjective ratings of withdrawal declined significantly, and the effects of administered heroin (measured by pupil diameter) did not begin to re-emerge until the fifth week. Krupitsky et al. (2011) also found support for the use of injectable naltrexone for opioid dependence. Compared to placebo (35%), depot naltrexone resulted in a 90% abstinence rate (confirmed by urine testing), -10.1% change in craving (vs. +0.7% in the placebo group), and longer treatment retention (168 days for naltrexone vs. 96 days for placebo).

Naltrexone and Alcohol

While opioid receptor antagonists are used in the treatment of opioid dependence, the first FDA-approved use for naltrexone was as a treatment for alcohol dependence. Srisurapanont and Jarusaraisin (2005) performed a meta-analysis of 24 studies (with a total of 2,861 human subjects) that assessed naltrexone for the treatment of alcoholism prior to 2005. They found that for short-term treatment, naltrexone did not decrease discontinuation of treatment, but did significantly decrease the incident of relapse. They concluded that, while the perfect duration length for treatment is not yet known, naltrexone should be strongly considered as a viable short-term treatment option for alcoholism, and some form of adjunct psychosocial therapy should be used with all patients receiving naltrexone in order to maximize positive treatment outcomes.

Deas, May, Randall, Johnson, and Anton (2005) examined naltrexone treatment outcomes in adolescent alcoholics in an open-label pilot study. The average number of daily drinks for subjects per drinking day decreased significantly from baseline over a six-week period, suggesting that naltrexone administration may result in significant

decreases in both craving and use. They found that alcohol-dependent adolescents tolerated naltrexone well, with the same minor side effects that occur in adults, and concluded that naltrexone would be a viable option in the treatment of adolescent alcohol disorders.

Peterson, Conrod, Vassileva, Gianoulakis, and Pihl (2006) studied the effects of naltrexone on the physiological, behavioral, and subjective effects of acute alcohol intoxication. They found that when alcohol was administered, naltrexone had a significant effect on blood-alcohol concentration, reduced the characteristic heart rate increase of alcohol intoxication, and accounted for changes in the subjects' subjective and behavioral responses to alcohol. A major finding was that naltrexone specifically blocked the stimulant properties of alcohol that characteristically appear during early acute intoxication.

Weerts et al. (2008) used positron emission tomography to measure opioid receptor blockade in 21 recently abstinent alcohol-dependent subjects who were being treated with naltrexone. They found almost complete blockade of mu-opioid receptors, along with partial blockade of delta-opioid receptors in all subjects. This finding is significant because these two subtypes of opioid receptors are thought to be instrumental in modulating the reinforcing properties of alcohol and the maintenance of alcohol consumption. Not coincidentally, it is known that rodent strains which show preference for alcohol display differences in receptor density for these two subtypes (Weerts et al., 2008). For instance, Marinelii, Kiianmaa, and Gianoulakis (2000) observed a significantly higher degree of mu-opioid receptor binding in the shell of the nucleus accumbens and in the prefrontal cortex of selectively bred AA (alko, alcohol) rats than in

ANA (alko, non-alcohol) rats. McBride, Chernet, McKinzie, Lumeng, and Li (1998) observed mu-opioid receptor density in alcohol-preferring rats to be approximately 20 % higher in the olfactory tubercle and the nucleus accumbens, 25 % higher in the basolateral and lateral nuclei of the amygdala, 15% higher in the caudate-putamen, and between 10 and 30 % *lower* in the hippocampus than in the non-alcohol-preferring strain. Soini, Ovaska, Honkanen, Hyttia, and Korpi (1998) found that overall delta-opioid receptor density was significantly lower in the brains of AA rats versus ANA rats. Soini and colleagues (1998) suggest that differences in receptor density and distribution in brain regions associated with cognition, emotion, and motivation may be neurochemical correlates of differences in alcohol-drinking behavior between different animal strains. However, McBride and colleagues (1998) note that opioid receptors are likely just one small part of a much larger and more complex system involved in mediating alcohol use, and caution that our current understanding of how alcohol use is mediated only allows for acknowledgement of a correlation between opioid receptor density and propensity for alcohol use.

In addition to trials with human subjects, the effects of naltrexone on alcohol consumption and effects has also been studied in laboratory animals. Varashin et al. (2005) studied the effects of the microinjection of naltrexone into the nucleus accumbens of rats. They found that pretreatment with naltrexone prevented the development of tolerance to the motor effects of alcohol, adding further evidence to the idea that the opioid system is involved in the development of tolerance to the effects of alcohol. Czachowski and Delory (2009) studied the treatment effects of naltrexone and acamprosate (a drug that is known to decrease glutamate levels and increase beta-

endorphin release [Kalk & Lingford-Huges, 2014]) on alcohol seeking and drinking versus sucrose seeking and drinking in alcohol-dependent and nondependent rats. They found that naltrexone decreased alcohol seeking and consumption in nondependent rats. The researchers hypothesized that the "nondependent" group could model early-stage problem drinking in humans, therefore suggesting that naltrexone could be used for purposes of intervention at this stage of drinking, in addition to relapse prevention for newly abstinent alcohol-dependent patients.

Naltrexone and Nicotine

In addition to its approved uses for the treatment of alcohol and opioid dependence, other studies have investigated naltrexone's effect on other drugs of abuse. While it has been established that nicotinic receptors and the dopamine system are involved in nicotine addiction (Gonzales, Rennard, Nides et al., 2006; Benowitz, 2010), the role of opioid receptors has also become an area of interest in nicotine research (Walters, Cleck, Kuo, & Blendy, 2005). As is the case with alcohol abuse and dependence, the opioid system is thought to play a major role in mediating the effects of stimulant drugs, and research has been conducted to investigate this assumption. For instance, Campbell, Taylor, and Tizabi (2007) found that the selective opioid antagonists D-Phe–Cys–Tyr–D-Trp–Arg–Thr–Pen–Thr-NH2 (CTAP), which is selective for mu-opioid receptors, naltrindole (selective for delta-opioid receptors), and norbinaltorphimine (selective for kappa-opioid receptors) were each successful in blocking the antinociceptive effects of nicotine and/or alcohol in Wistar rats. Jackson et al. (2010) similarly found that pretreatment with the highly selective kappa-opioid receptor antagonist JDTic ((3R)-7-hydroxy-N-((1S)-1-[[(3R,4R)-4-(3-hydroxyphenyl)-

3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide) attenuated physical and affective signs of nicotine withdrawal in mice, and concluded that kappa-opioid receptors are clearly involved in mediating nicotine withdrawal symptoms. This builds upon the findings of Todtenkopf, Marcus, Portoghese, and Carlezon (2004) that the stimulation of kappa-opioid receptors in the brain triggers depressive-like symptoms in animal subjects, as well as Todtenkopf and colleagues' (2004) suggestion that kappa-opioid antagonists may have efficacy as antidepressants.

In addition to the aforementioned opioid receptor antagonists, the effect of naltrexone on nicotine use has also been studied. For instance, Krishnan-Sarin, Meandzija, and O'Malley (2003) found in a small-sample preliminary study that naltrexone showed potential as a very promising treatment option: it decreased incident of relapse and reduced smokers' desires to smoke tobacco. King and Meyer (2000) found that nicotine-dependent subjects reported reduced cravings and desire to smoke when given oral naltrexone. Rustkalis et al. (2005) studied the effect of naltrexone pretreatment on human cigarette smokers. They found that the relative reinforcing value of nicotine via cigarette smoking was significantly lower following administration of naltrexone, and concluded that further investigation of opioid receptor antagonists as a possible component of smoking cessation programs is warranted based on these results. Rohsenow et al. (2007) concluded that higher-dose transdermal nicotine replacement therapy (i.e., the "nicotine patch") was a more promising smoking cessation treatment than naltrexone, but other subsequent studies have shown further support for the use of naltrexone in the treatment of nicotine addiction.

Liu et al. (2009) studied whether treatment with naltrexone has an effect on the conditioned incentive salience of nicotine cues in rats. They found that naltrexone attenuated cue-induced reinstatement of nicotine-seeking after extinction had taken place, but that naltrexone did not affect nicotine self-administration behavior. These results indicate that naltrexone may be useful in the prevention of cue-induced relapse to cigarette smoking in recently abstinent nicotine addicts. This discrepancy may also indicate that the conditioned place preference paradigm has higher ecological validity as an approximation of human nicotine-seeking behavior than the self-administration paradigm.

Other studies have examined the effect of naltrexone on nicotine in the context of alcohol use. For instance, Ray et al. (2007) studied the effects of alcohol and naltrexone on nicotine craving in light smokers. They found that while the pharmacological effects of alcohol itself induced craving for nicotine, naltrexone greatly lessens the cigarette craving during alcohol use. These researchers concluded that naltrexone is effective for reducing smoking in heavy drinkers. Likewise, O'Malley et al. (2009) studied the effect of naltrexone therapy on the reduction of hazardous alcohol use by subjects during a trial of naltrexone for smoking cessation. Their findings suggest that naltrexone may be able to lessen the risk of hazardous drinking in people who both drink alcohol and smoke cigarettes and are not attempting to reduce their alcohol consumption (by treatment enrollment or otherwise).

Nicotine and Incentive Salience

There are several different aspects to, or types of, reward that are involved in drug addiction. One type of reward can be called hedonic pleasure, or hedonic impact, which

refers to "liking" something-- in other words, finding a food, drug, or activity to be palatable or subjectively pleasurable upon experiencing it. Another aspect of reward is incentive salience, which, in the case of drug use and dependence, involves associating external stimuli, or cues, with taking the drug, causing a "wanting" (Zhang, Berridge, Tindell, Smith, & Aldridge, (2009).

This is important in the study of drug addiction for several reasons. Hedonic "liking" is often associated with drug use or abuse, where a person (or laboratory animal) engages in use of a drug for its pleasurable effects. "Wanting," on the other hand, which is where incentive salience comes in, is characteristic of experienced drug users who already have assigned a positive valence to the drug and are able to make predictions about what will happen when they obtain the drug and self-administer it. This "wanting" can result in motivation to continue using a drug even if the drug's hedonic impact ("liking") has been suppressed by tolerance. Additionally, "wanting" may continue to persist when drug-related cues are present even if predictions about the drug's value have become negatively valenced, such as in the case of "recovering addicts" who have experienced significant adverse consequences of their drug use (Smith, Berridge, & Aldridge, 2011).

A basic way to describe incentive salience is to say that it is a motivational property that involves an intense "pulse" of "wanting" that is triggered by a cue, and is modulated by internal physiological states such as drug withdrawal (Zhang, Berridge, Tindell, Smith, & Aldridge, 2009). For chronic tobacco smokers, this may appear as wanting tobacco in situations or circumstances when they normally smoke, as nicotine is

very effective in establishing or magnifying the incentive and reinforcing properties of environmental stimuli associated with smoking (Caggiula et al., 2001).

Smith, Berridge, and Aldridge (2011) investigated signals for these different aspects of reward in the nucleus accumbens and ventral palladium in rats, using microinjections of drugs that stimulated either opioid or dopamine receptors. They concluded that signals for hedonic pleasure (liking) and incentive salience (wanting/motivation), as well as Pavlovian prediction (learning), are all separate and distinguishable processes in the mesocorticolimbic circuits of the nucleus accumbens and ventral palladium. Stimulation of the dopamine system increased motivation (wanting), but did not affect liking or learning signals. Stimulation of the opioid system, on the other hand, increased both liking signals *and* incentive salience signals. The fact that there is evidence for separate, distinguishable pathways in the brain for each of these aspects of drug reward, and evidence that different neurotransmitters may play specific roles in each aspect of reward is an exciting revelation that has significant implications for nicotine-related pharmacotherapy research. Additionally, this study implies that the opioid system may play just as large a role in mediating nicotine addiction as does the dopamine system, which would help to explain and further validate the results of the previously mentioned studies on the effect of naltrexone on nicotine dependence.

Conditioned Place Preference

A common procedure used to measure the behavioral effects of drugs in laboratory animals is conditioned place preference, which involves assigning motivational properties to specific "places," via reward pairing. In this paradigm, the stimuli within a specific environment, or place, become associated with a drug's

reinforcing effects through repeatedly pairing the drug's reinforcing effects with the environment. A "place preference" is evidenced by the animal spending a larger amount of time in the reward-paired environment, or place, than it did previous to the association of that environment with the reward (Calcagnetti & Schechter, 1994). Thus, it has been speculated that when a drug is rewarding to an animal, a specific place that has been paired with that drug may acquire an incentive motivational property that results in the animal "preferring" it to places that have not been paired with the drug (Flagel, Akil, & Robinson, 2009).

A typical conditioned place preference procedure involves administering a drug to a subject (i.e. - via injection) and confining it to one stimulus-distinct compartment of a shuttlebox, which consists of two or three compartments and is normally used for conditioned place preference procedures in rodents. This is repeated daily, with the caveat that every other day the animal is administered a control (i.e. - saline injection without the drug) and placed in the stimulus-distinct compartment on the opposite side of the apparatus. Then, during the testing phase, the animal is not given the drug and the researcher measures how much time the animal spends in each compartment. If the animal spends more time in the compartment where the drug was administered, it can be inferred that the animal "likes" the drug and prefers to spend time in the compartment that it associates with the effects of the drug. In this case, conditioned place *preference* is said to have occurred. It is also possible that the animal found the effects of the drug aversive, and may choose to spend *less* time in the compartment associated with the drug. If this occurs, it is known as conditioned place *aversion* (Calcagnetti & Schechter, 1994).

Conditioned Place Preference with Nicotine

Numerous studies have evaluated nicotine in the conditioned place preference paradigm. Conditioned place preference with nicotine dates back at least as far as Fudala et al (1985) and Fudala and Iwamoto (1986), who found that nicotine induced conditioned place preference in rats. After a number of additional studies by other researchers in the late 1980s and early 1990s produced conflicting results in demonstrating place preference for nicotine, Calcagnetti and Schechter (1994) focused on conducting a conditioned place preference experiment with a biased approach that utilized baseline preference assessments prior to drug pairing. With the biased approach, the drug being studied is paired with each individual animal's less-preferred shuttlebox chamber; thus it takes into account individual differences between subjects, which is thought to increase both the validity and the reliability of the conditioned place preference paradigm.

Walters, Brown, Changeaux, Martin, and Damaj (2006) further investigated the mechanisms underlying the reinforcing properties of nicotine using a conditioned place preference paradigm in wild-type mice. They found that pretreatment with the [alpha]-4-[beta]-2 subunit of the nicotinic acetylcholine receptor antagonist dihydro-[beta]-erythroidine blocked conditioned place preference for nicotine in their animal subjects.

In addition to nicotinic receptors, opioid receptors may also play a role in nicotine's conditioned place preference effects. For instance, Walters et al. (2005) used conditioned place preference in an experiment that looked at the relationship between nicotine, naloxone, and the transcription factor CREB. Walters and colleagues (2005) further investigated previous findings that nicotine causes a release of endogenous

opioids in different parts of the brain in rats and mice, and found that a single administration of the opioid-receptor antagonist naloxone blocked the conditioned behavioral response of nicotine reward in a conditioned place preference paradigm. Zarrindast, Faraji, Rostami, Sahraei, and Ghoshouni (2003) found that naloxone blocked place preference for both nicotine and morphine in mice.

Smith et al. (2012) specifically investigated the involvement of the dynorphin/kappa-opioid system in nicotine addiction. They found that nicotine indeed produces conditioned place preference in mice, and found that place preference for nicotine was blocked by the opioid receptor antagonist norbinaltorphimine. They also stress the validity of comparing conditioned place preference in animal models to nicotine-related human behaviors by pointing out that activation of the kappa-opioid system elicits similar dysphoric responses and anxious behaviors in both humans and rodents.

Rationale

The opioid-receptor antagonist naltrexone, which is approved as a pharmacotherapy for the treatment of opioid dependence and alcohol dependence, has more recently been examined as a possible treatment for dependence on other drugs of abuse. As we have seen in the preceding sections, there exists compelling evidence that opioid receptors play a role in mediating the conditioned place preference effects of nicotine, and it has been demonstrated that the opioid receptor-antagonist naloxone can block place preference for nicotine. Since naltrexone has proven promising in the treatment of dependence on other drugs, we believe that further evaluation of naltrexone

as a pharmacotherapy for nicotine dependence is warranted, and the best way to do this in an animal model is by utilizing the conditioned place preference paradigm.

Method

Experiment 1

Subjects. Forty male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) weighing at least 250 g were used for this experiment. Rats were group housed and provided food and water ad lib in their home cages. All procedures were approved by the Institutional Animal Care and Use Committee for Northern Michigan University and followed the Guide for the Care and Use of Laboratory Animals, 2nd edition (2012). Animals were kept on a 12-hour light/dark cycle (lights on at 11:00 a.m. and off at 11:00 p.m.), and were housed in a temperature-controlled room adjacent to the testing room.

Drugs. Both naltrexone hydrochloride (Sigma-Aldrich, St. Louis, MO) and nicotine hydrochloride (Sigma-Aldrich) were dissolved in a 0.9% physiological saline solution. Drugs were administered subcutaneously in a 1 ml/kg volume. All doses refer to the salt form for these drugs. Nicotine was administered 10 minutes and naltrexone was administered 15 minutes before animals were placed into the apparatus for conditioning.

Apparatus. Two standard two-chamber shuttleboxes (18.4 cm x 22.9 cm x 16.5 cm for each chamber), constructed with stainless steel and Plexiglas, with tilting grid floors were used (Med-Associates Inc., St. Albans, VT). Each shuttle box featured a guillotine door between the two chambers that could be opened or remain closed as required by the experimenter. The left-hand chambers' floors consisted of bare horizontal stainless steel floor bars, while the right-hand chambers' floors had wire-

screen flooring on top of the floor bars to give each chamber a different tactile stimulus. The bedding pans for the left-hand chambers contained corncob bedding, and the right-hand chambers' bedding pans contained pine shavings, giving each chamber a different odor. The left-hand chambers' clear Plexiglas walls and ceilings were covered with black cloth hoods, while the walls of the right-hand chambers were not covered or altered at all, thus giving each chamber different visual stimuli. Shuttle boxes were housed in sound-attenuated cubicles equipped with fans for ventilation and masking noise. All chambers were lit by 20 amp interior bulbs during all conditioning and testing sessions. Data were collected using Med PC version 4 for Windows (Med-Associates Inc.).

Procedure. In this experiment, a total of 40 animals were assigned to 5 groups, based on an assigned drug condition, with each group consisting of 8 animals. Animals were selected for each group (N = 8) in a counter balanced manner by body weight. The drug conditions were nicotine (0.8 mg/kg), naltrexone (1.0 mg/kg), and naltrexone (3.0 mg/kg), as well as two 0.9% physiological saline conditions, which served as the vehicle controls for nicotine and naltrexone, respectively. The nicotine dose was selected based upon the work of Calcagnetti and Schechter (1994), who observed a place preference for nicotine in male Sprague-Dawley rats at this dosage. Naltrexone dosages used in this study were selected based upon the methods and findings of several previous studies involving naltrexone and rats, including Olmstead and Burns (2005), Varashin, Wazlawik, and Morato (2005), Czachowski and Delory (2009), and Liu et al. (2009).

The first phase of the conditioned place preference procedure consisted of acclimating animals to the study environment by bringing the animals' home cages into the testing environment for 2 hours a day for two consecutive days. Next, the animals

were allowed to freely explore both chambers of the shuttle box during 10-minute "baseline" sessions for 3 consecutive days. The amount of time spent on each side was recorded in order to determine each animal's preferred compartment. Preference was determined by recording the number of seconds spent by each animal per compartment and then taking the mean of these times across the three baseline sessions.

Following this, 30-minute drug and saline pairing sessions were conducted for 8 consecutive days, with saline (control) or drug given on alternating days. The doors between chambers were closed at all times during pairing sessions so that each animal remained in the intended chamber. On "control" days, each animal was placed in its preferred chamber and on drug days, each animal was placed in its non-preferred chamber. For animals assigned to the saline treatment condition, as a control group for either nicotine or naltrexone, saline was given every day in the same alternating fashion (i.e., saline was pairing with both the preferred and non-preferred chamber). This is consistent with the biased conditioned place preference procedure set forth by Calcagnetti and Schechter (1994), in which the drug is paired with the animal's non-preferred chamber as determined by baseline measurements (as opposed to the unbiased procedure, in which no baseline preference is determined and drugs are paired with chambers as determined by the researcher) On the day immediately following the final pairing session, a test session was conducted, which consisted of allowing the animals to freely explore the shuttle box with the guillotine door open for 10 minutes with no pre-session injection.

In Experiment 1, ten animals were studied at a time. For instance, animals tail-marked "1" through "10" were run through the experiment first (animals were assigned

identification numbers arbitrarily), followed by animals "11" through "20," and so forth. This was done due to the time constraints of the researcher's schedule, and the fact that testing all animals in the experiment simultaneously would have been unfeasible with only two shuttleboxes. Shuttleboxes were cleaned at the end of each daily session, and were spot-cleaned as needed between animals (for instance, if an animal defecated while in the shuttlebox, the feces would be removed before the next animal entered). This was the standard procedure during all baseline, drug pairing, and testing sessions.

Data Analysis. The dependent variables for this study consisted of time (s) spent in the non-preferred compartment and a percentage based on the time spent in the non-preferred compartment after pairing compared to time spent in the non-preferred compartment before pairing. Data were calculated as means (+/- standard error of the mean [SEM]). A between groups analysis of variance (ANOVA) was conducted for naltrexone, an independent samples t test was conducted for nicotine, and a dependent samples t-test was used to compare preference before pairing vs. preference after pairing. All analyses were conducted using GraphPad Prism version 6.0 for Windows (GraphPad Prism, La Jolla, CA).

Experiment 2

Subjects. This experiment was conducted following an inability to establish a conditioned place preference for nicotine in experiment 1. Twenty rats were used in Experiment 2, and the animals were assigned to a group that received nicotine (0.8 mg/kg) on drug conditioning days or to a control group that received saline every day during conditioning. All other animal care conditions were the same as in experiment 1.

Drugs. Only nicotine hydrochloride (0.8 mg/kg; Sigma-Aldrich) and 0.9% physiological saline were used in experiment 2. See experiment 1 for further information.

Apparatus. The shuttle boxes used in experiment 1 were used for this experiment. However, in this experiment, the black-hood that covered the left-hand chamber was replaced with vertical black lines on the walls and no covering on the ceiling. The black lines were printed on white paper, which was trimmed to fit the shuttle box walls. See experiment 1 for all other details for this apparatus.

Procedure. The purpose of this experiment was to determine if we could establish a conditioned place preference for nicotine by adjusting the appearance of the left-hand compartment. Otherwise, the procedures described in experiment 1 were identical those used for experiment 2.

Data Analysis. The data analysis procedures described in experiment 1 were identical to those used for experiment 2.

Experiment 3

Subjects. This experiment was performed after a conditioned place preference for nicotine was found in experiment 2, which used a different appearance for the non-preferred compartment than in experiment 1. Forty animals were used for experiment 3, and were assigned to the following four conditions (N = 10): naltrexone (3.0 mg/kg) + saline, saline + nicotine (0.8 mg/kg), saline + saline, and naltrexone (3.0 mg/kg) + nicotine (0.8 mg/kg). All animal care conditions were the same as used in Experiments 1 and 2.

Drugs. Nicotine hydrochloride (0.8 mg/kg; Sigma-Aldrich), naltrexone hydrochloride (3.0 mg/kg; Sigma-Aldrich), and 0.9% physiological saline were used in experiment 3. Pretreatment times were identical to those in the first two experiments (15 minutes for naltrexone, and 10 minutes for nicotine). For instance, in the case of the "naltrexone + nicotine" group, naltrexone was administered 15 minutes prior to being placed into the shuttle box for training, with nicotine then being administered 5 minutes after the naltrexone injection (10 minutes before being placed into the shuttle box). In the "saline + nicotine" group, saline took the place of naltrexone and was administered 15 minutes prior to placing the subject in the shuttle box; in the "naltrexone + saline" group, saline took the place of nicotine and was administered 10 minutes prior to the training session. In the "saline + saline" group, the first saline injection was given in lieu of naltrexone (15 minutes before training initiation), and the second saline injection was given in lieu of nicotine (administered at the 10-minute mark). See Experiment 1 for further information.

Apparatus. All apparatus conditions in this experiment were identical to those in Experiment 2.

Procedure. The conditioned place preference procedures used for experiment 3 were the same as those used for experiments 1 and 2.

Data Analysis. A one-way between groups ANOVA was used to assess potential differences between these treatment conditions. Otherwise, the data analysis procedures used for experiment 3 were same used for experiment 1.

Results

Experiment 1

Baseline. During the 600-second baseline sessions, subjects spent a mean of 289.67 s (+/- SEM=12.74) (48.28% of the total time) in the left-hand chamber and a mean of 297.64 s (+/- SEM = 12.81) (49.61% of the total time) in the right-hand chamber per session (time spent in the doorway between the two chambers accounts for the remaining time/percent). After their preferred and non-preferred sides were determined, it was calculated that each subject spent a mean of 361.95 s (+/- SEM =6.96) (60.33% of the total time) on its preferred side and an average of 226.19 s (+/- SEM = 6.69) (37.70% of the total time) on its non-preferred side per baseline session. Of the 40 subjects used in this experiment, 20 (50%) of them preferred the left-hand chamber and 20 (50%) preferred the right-hand chamber.

Nicotine Conditioned Place Preference. The data for conditioned place preference conducted with nicotine are shown in Figure 1. During the test session following training, a statistical difference was not found between the number of seconds spent in the saline-paired compartment (M= 304.90 s +/- SEM= 30.89) versus the nicotine-paired compartment (M = 263.50 s +/- SEM = 35.64), $t(14) = 0.88$, $p > 0.05$. A statistical difference was also not found when comparing the mean time spent in the non-preferred compartment during baseline sessions (M = 223.80 s +/- SEM = 12.62) to the mean time spent in the non-preferred compartment after pairing with nicotine (M = 263.5 s +/- SEM = 35.64), $t (7) = 1.19$, $p > 0.05$. Finally, an analysis was conducted to determine if the percentage of time spent in the non-preferred side after pairing compared to time spent in the non-preferred side before pairing in saline-treated rats versus

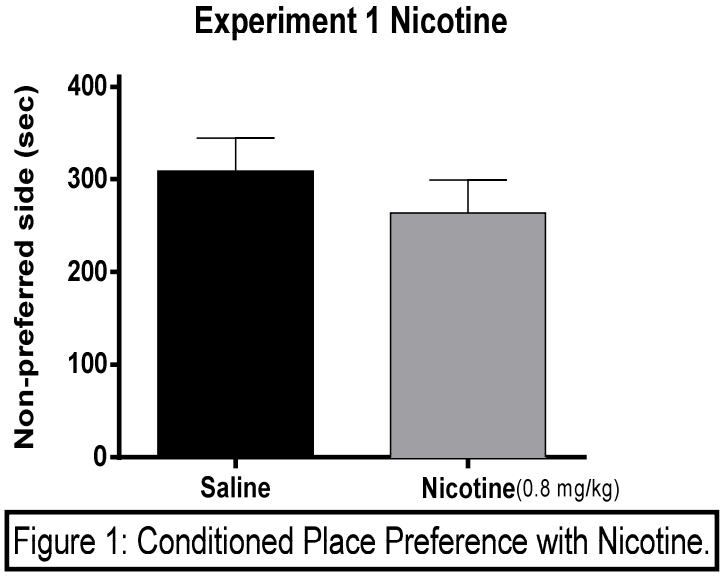


Figure 1: Conditioned Place Preference with Nicotine.

nicotine-treated rats. A statistically significant difference was not found between the saline-treated rats (M = 153.60% +/- SEM = 20.54) compared to the nicotine-treated rats (M = 119.10% +/- SEM = 15.78), t (14) = 1.33, p > 0.05.

Naltrexone Conditioned Place Preference. The data for conditioned place preference conducted with naltrexone are shown in Figure 2. Statistically significant differences were not found for the time spent in the non-preferred side between the saline (M = 253.60 +/- SEM =21.78), naltrexone 1.0 mg/kg (M = 233.80 +/- SEM = 34.48), and naltrexone 3.0 mg/kg (M = 214.40+/- SEM = 40.65) treatment groups, $F(2, 21) = 0.35$, $p = 0.710$. Also, a statistical difference was not found when comparing the mean time spent in the non-preferred compartment during baseline sessions (M = 243.30 s +/- SEM = 18.52) compared to number of seconds spent in the non-preferred compartment after pairing with naltrexone 1.0 (M = 233.80s +/- SEM = 34.48), t(7) = 0.25, p > 0.05. Likewise, a statistical difference was not found when comparing the mean time spent in the non-preferred compartment during baseline sessions (M = 236.00 s +/- SEM = 16.85)

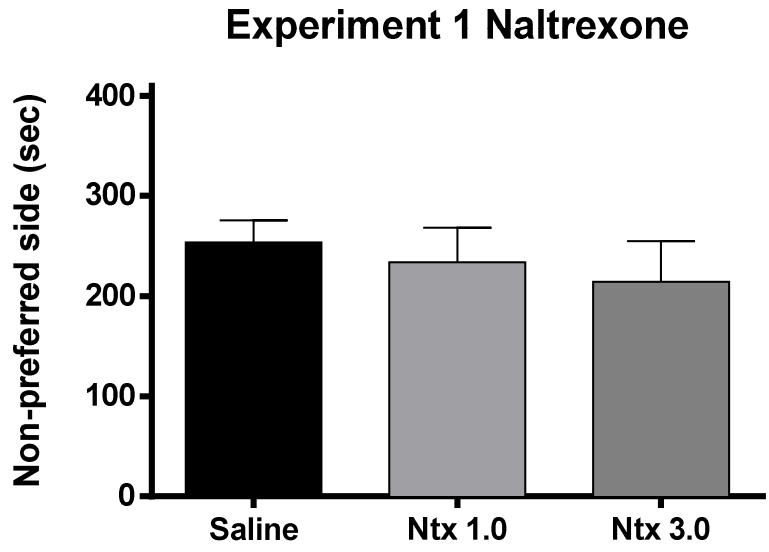


Figure 2: Conditioned Place Preference with Naltrexone

compared to number of seconds spent in the non-preferred compartment after pairing with naltrexone 3.0 (M = 214.40 s +/- SEM = 40.65), t (7) = 0.54, p > 0.05. Finally, an analysis of variance was conducted on the percent change in time spent in the non-preferred side from before pairing to after pairing for rats in the saline group (M = 117.00% +/- SEM = 10.75), the naltrexone 1.0 group (M = 101.20% +/- SEM = 17.46), and the naltrexone 3.0 group (M = 93.72% +/- SEM = 19.46). The results of the analysis did not show a statistically significant difference, F (2, 21) = 0.53, p = 0.595.

Experiment 2

Baseline. During the baseline sessions, subjects spent an average of 243.88 s (+/- SEM = 15.93) (40.65% of the time) in the left-hand chamber and an average of 342.87 s (+/- SEM = 15.95) (57.14%) in the right-hand chamber. After their preferred and non-preferred sides were determined, it was calculated that each subject spent an average of 361.15 s (+/- SEM = 10.93) (60.19%) on its preferred side and an average of 225.45 s (+/- SEM = 11.05) (37.58%) on its non-preferred side per baseline session. Out of the 20

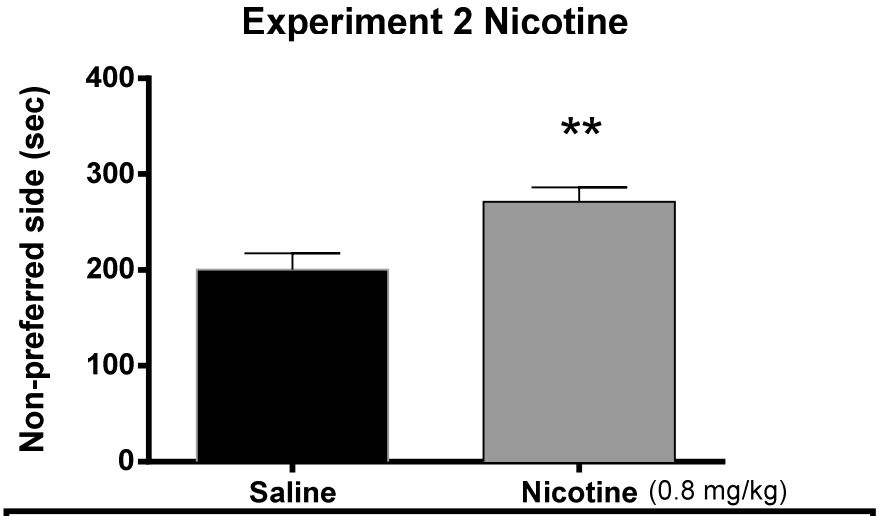


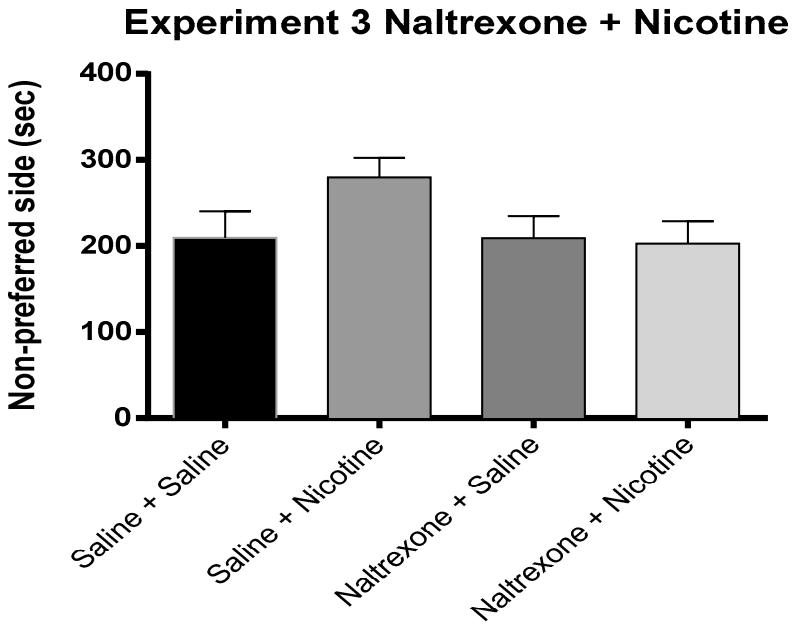
Figure 3: Conditioned Place Preference with Nicotine

subjects used in this experiment, 5 (25%) of them preferred the left-hand chamber and 15 (75%) preferred the right-hand chamber.

Nicotine Conditioned Place Preference. The data for conditioned place preference conducted with nicotine are shown in Figure 3. During the test session following training, the number of seconds spent in the saline-paired compartment (M= 200.40 s +/- SEM= 17.17) was significantly less the number of seconds spent in the nicotine-paired compartment (M= 271.00 s +/- SEM = 15.31), $t(18) = 3.07$, $p < 0.05$. The mean time spent in the non-preferred compartment during baseline sessions (M = 216.50 s +/- SEM = 19.31) was significantly less than the amount of time spent in the non-preferred compartment after pairing with nicotine (M = 271.00 s +/- SEM = 15.31), $t(18) = 2.21$, $p < 0.05$.

Experiment 3

Baseline. During the baseline sessions, subjects spent an average of 262.86 s (+/- SEM = 6.98) (43.81% of the time) in the left-hand chamber and an average of 324.08 s



Note: Nicotine (0.8 mg/kg), Naltrexone (3.0 mg/kg) used in Experiment 3.

Figure 4: Conditioned Place Preference with Naltrexone + Nicotine Combination

(+/- SEM = 6.77) (54.01%) in the right-hand chamber per session. After their preferred and non-preferred sides were determined, it was calculated that each subject spent an average of 336.80 s (+/- SEM = 4.79) (56.13%) on its preferred side and an average of 250.2 s (+/- SEM = 4.84) (41.70%) on its non-preferred side per baseline session. Of the 40 subjects used in this experiment, 7 (17.5%) of them preferred the left-hand chamber and 33 (82.5%) preferred the right-hand chamber.

Naltrexone + Nicotine. The data for conditioned place preference conducted with nicotine paired with naltrexone are shown in Figure 4. An analysis of variance did not show a significant difference for the number of seconds spent in the non-preferred side between the treatment groups, $F(3, 36) = 1.505$, $p = 0.230$. A mixed two-factor analysis of analysis of variance using training (before and after) as a within subjects factor and treatment group as a between groups factor revealed a statistically significant

increase in time spent in the nonpreferred compartment after training compared to before training ($F[1,35] = 4.22$, $p = 0.048$), but no statistically significant differences for the treatment factor ($F[3, 35] = 1.95$, $p = 0.140$) or an interaction effect ($F[3, 35] = 1.48$, $p = 0.089$).

Discussion

The present study reported on the effects of the opioid receptor antagonist naltrexone on the ability of nicotine to produce a conditioned place preference, a model for the incentive salience theory of drug addiction. In the first experiment, acute repeated administration of nicotine did not produce a statistically significant place preference, and acute repeated administration of two different doses of naltrexone also did not produce a statistically significant place preference. In the second experiment, after altering the visual stimuli in the shuttle boxes, acute repeated administration of nicotine did produce a statistically significant conditioned place preference. In the third experiment nicotine failed to produce a significant place preference; thus, a potential reversal of a nicotine place preference by naltrexone could not be evaluated.

The potential to demonstrate a conditioned place preference for nicotine in rodents has previously been established in several studies, including Calcagnetti and Schechter (1994), who used male Sprague-Dawley rats (as we chose to use in the present study), as well as by Walters et al. (2005), Walters et al. (2006), and Smith et al. (2012), who all used mice. However, Calcagnetti and Schechter (1994) stated that nicotine may not produce conditioned place preference as reliably as opioids or as other stimulant drugs do, noting that a few studies from the 1980s and early 1990s either failed to demonstrate nicotine place preference or found a nicotine place aversion. This

unpredictability has been echoed more recently by Natarajan, Wright, and Harding (2011), who note that studies throughout the early 2000s have also produced conflicting results on the ability of nicotine to produce a conditioned place preference. Also relevant to this discussion is the idea of pretreating animals with drugs to develop tolerance prior to an experiment. This practice, in theory, would make the studies with animal subjects more closely analogous to humans that were heavy smokers. Zarrindast et al. (2003) used a different approach for studying nicotine place preference by treating mice with nicotine daily for 12 consecutive days prior to conducting conditioned place preference procedures in order to establish a tolerance to the aversive effects of nicotine prior to initiating the conditioned place preference procedure. They were able to establish a place pereference with nicotine and were able to block the nicotine place preference with naloxone. Thus, future research involving nicotine and a conditioned place preference paradigm may wish to consider this option.

Also, previous studies have used a variety of different place preference apparatus setups, with some choosing to use three-compartment shuttle boxes (and thus employing an unforced choice procedure), and other studies utilized the forced-choice procedure similar to the two-compartment shuttle boxes used in the present study. For example, Calcagnetti and Schechter (1994), Walters, et al. (2006), Natarajan, Wright, and Harding (2011), and Smith, et al. (2012) all used three-compartment shuttle boxes, while Zarrindast, et al. (2003) and Walters, et al. (2005) used a two-compartment apparatus (nearly identical to the one we employed in this study), and Olmstead and Burns (2005) used a two-compartment apparatus with a tunnel between the compartments. It should be noted that there appears to be no consensus on which type of place preference apparatus

configuration is the best option to use for conditioned place preference. However, the primary concern with the forced choice procedure (as we employed in the current study) is the potential of unintentionally or unknowingly creating a bias for the side of the shuttlebox that an animal is placed into at the beginning of the testing session (Prus, James, & Rosecrans, 2009). This issue was not anticipated or controlled for in the current study, so it is possible that a compartment bias due to initial placement may have confounded the results, and future research in this area may wish to strongly consider employing an unforced choice procedure instead to avoid this potential bias.

Prior to Experiment 1, we had decided to use a black cloth hood over the Plexiglas walls and ceiling of one chamber, with nothing covering the clear walls and ceiling of the opposite chamber. This configuration was similar to several previous conditioned place preference studies. For instance, Calcagnetti and Schechter (1994) used a white light bulb and had metal bars on the floor in one chamber (much like the apparatus used in the present study), but used a red light bulb and a black plastic floor in the opposite chamber to make it darker, and Natarajan, Wright, and Harding (2011) employed a wooden shuttle box with one main compartment painted white and the other painted black.

However, after the completion of Experiment 1, in which nicotine did not establish a conditioned place preference, we decided to re-evaluate our methods. Upon re-examining a variety of conditioned place preference literature, we discovered that one phenomenon that can be of concern to researchers in conditioned place preference studies is that of compartment preference bias, in which animals tend to prefer one compartment over the other due to its' environment being noticeably more "comfortable" to them. For

instance, in Zarrindast and colleagues' 2003 study, which employed an apparatus with one compartment painted white and the other black, all animals preferred the black side. However we did not have statistically significant evidence of compartment bias in Experiment 1. In fact, the opposite seemed to be true, as the left-side (50%) vs. right-side (50%) baseline preferences were perfectly *unbiased*. This led a concern that perhaps the stimuli in each compartment were not distinct enough, and thus the animals were not able to effectively discriminate between compartments (essentially the exact opposite of compartment preference bias).

Thus, we decided that it would be prudent to consider some of the different methods used in previous conditioned place preference studies to see if we could find an alternative that would increase the stimulus distinction between the two chambers. Smith et al. (2012) utilized the same setup for their three-compartment shuttle box as Schindler, Li, and Chavkin (2010), in which one main compartment had vertically-oriented alternating white and black stripes and the opposite chamber had horizontal-oriented stripes, with the center chamber painted plain white. We decided to use a modified version of this white-and-black-stripes stimulus for our two-chamber shuttle boxes, similar to what Olmstead and Burns (2005) used in their study involving opioids and conditioned place preference (see Methods section for further details). Our subsequent demonstration of a place preference for nicotine in Experiment 2 led to the use of this equipment set up for experiment 3. We cannot conclusively state whether or not altering the visual stimulus on one side of our shuttle boxes directly led to establishing conditioned place preference in Experiment 2, but it is, at the very least, a noteworthy coincidence.

Walters et al. (2005) found that conditioned place preference for nicotine could be blocked in kappa-opioid receptor knockout mice by pretreatment with the opioid receptor antagonist naloxone on the test day following conditioning. Smith et al. (2012) were able to block an increase in place preference for nicotine in mice following forced swim stress with the opioid receptor antagonist norbinaltorphimine, although they found that norbinaltorphimine did not block nicotine place preference in animals not subjected to stress prior to testing. We were unable to demonstrate the ability of naltrexone to block place preference for nicotine in this experiment due to the lack of a significant place preference effect by nicotine in experiment 3. It is possible that our findings may be due to our particular combination of opioid receptor antagonist (naltrexone), rodent (Sprague-Dawley rats), type of apparatus (two-chamber forced-choice), and lack of any forced stress method prior to testing. Another possibility is that the nicotine dosage of 0.8 mg/kg may have been too low – we felt that our observation of nicotine place preference at that dosage in Experiment 2 indicated that it was adequate, but we did not find a place preference at that dosage in two out of the three experiments. Additionally, it is possible that having larger group sizes, and thus increased statistical power, could have resulted in statistically significant differences between groups in Experiment 3.

The idea of using manipulative techniques such as forced stress in order to obtain a preference for nicotine echoes the finding in self-administration studies that rats will not learn to self-administer nicotine acutely due to the drug's initial adverse effects (Prus, 2014). For instance, in order to induce nicotine self-administration, Boules, Oliveros, Liang, et al. (2011) first trained rats to obtain sucrose pellets via lever-pressing before exchanging the sucrose reward with nicotine injections. Another variable to consider in

nicotine research is the fact that, in addition to nicotine, tobacco also contains MAO inhibitors that are thought be instrumental in enhancing the rewarding effects of nicotine by increasing dopamine levels in the nucleus accumbens. For instance, Villegier, Lotfipour, McQuown, et al. (2007) found that rats self-administered nicotine after being injected with the non-selective MAO inhibitor tranylcypromine, and further research by Villegier, Belluzzi, and Leslie (2011) suggests that MAO inhibition may also increase serotonin levels.

Interestingly, our findings do somewhat parallel those of Liu et al. (2009), who found that while naltrexone did attenuate post-extinction reinstatement of nicotine-seeking in rats in a self-administration paradigm, it did not acutely affect self-administration behavior. As it relates to clinical applications, the findings of Liu et al. (2009) would seem to suggest that naltrexone may be more efficacious as a "relapse prevention" treatment for abstinent former tobacco users than as an intervention for current tobacco users. This idea is supported by the findings of Jackson, et al. (2010), who concluded that kappa opioid receptors are clearly involved in mediating nicotine withdrawal symptoms. These findings also fall in line with the findings of studies involving naltrexone's effect on other non-opioid drugs of abuse. For instance, Weerts et al. (2008) found that naltrexone almost completely inhibited mu-opioid receptors in their study of recently abstinent alcohol-dependent humans (a stage of the treatment process during which relapse prevention is the primary concern). Likewise, Srisurapanont and Jarusaraisin's (2005) meta-analysis found that while naltrexone did not decrease discontinuation of substance abuse treatment for alcohol-addicted humans, it did significantly decrease the incident of relapse. The finding that opioid receptors appear to

be involved in the expression of withdrawal symptoms in non-opioid drugs of abuse in animals models, along with indications that naltrexone may be effective as a smoking cessation aid in studies of human subjects (King & Meyer, 2000; Krishnan-Sarin, Meandzija, & O'Malley, 2003; Rustkalis et al., 2005; Ray et al., 2007) make this an intriguing area of study that has yet to be fully explored.

Conclusion

In summary, the purpose of the present study was to further investigate the effect of opioid receptor antagonism on nicotine in animal subjects. A conditioned place preference procedure was employed because this paradigm is thought to be an indicator of incentive salience, which is thought to be an important factor in the withdrawal symptoms experienced by recently abstinent tobacco users suffering from nicotine abstinence syndrome. After not finding a place preference for nicotine in the first experiment, a place preference for nicotine was established after changing the appearance of the experimental chamber in the second experiment. However, this configuration did not lead to a significant place preference for nicotine in the third experiment. Thus, the present study was not able to demonstrate any significant effects of naltrexone on nicotine-induced place preference.

While our results from the current study did not find any direct support for this idea, previous research suggests that opioid receptor antagonists may be a potential treatment for nicotine addiction. An advantage of evaluating opioid receptor antagonists is that two of these compounds (naloxone and naltrexone) are already FDA-approved for use in humans with opioid addiction and lead to relatively few adverse effects in humans. While clinicians may attempt to use naltrexone off-label for treating nicotine addiction,

the FDA will likely not allow clinical trials for opioid antagonist use in nicotine addiction without supporting data from animal models. Previous literature indicates that there are alternative approaches to studying nicotine in a conditioned place preference procedure and these other procedures should be examined in future studies.

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APPENDIX A

SIGNATURE PAGE

IACUC #: 238 **PROPOSAL TITLE (From cover page):** Conditioned place preference with nicotine and naltrexone 2

X. ACKNOWLEDGEMENT BY PRINCIPLE INVESTIGATOR

I acknowledge responsibility for this project. I have read the Northern Michigan University Principles for the Care and Use of Laboratory Animals and certify that this project will be conducted in compliance with those principles. I assure that I will obtain Institutional Animal Care and Use Committee approval prior to significant changes in the protocol. I assure that this project does not unnecessarily duplicate previous research or instructional projects. I assure that students, staff and faculty on the project are qualified or will be trained to conduct the project in a humane, safe, and scientific manner.

Signature: _____ 01/31/2014

Principal Investigator Date

XI. APPROVAL OF SCIENTIFIC MERIT (to be completed by the Department Head)

Before it is initiated, this project must be reviewed and approved on the basis of its scientific merit.

☐ Review conducted by external agency.

☐ Governmental Agency: Please specify the reviewing agency or board Federal agency (e.g., NIH, NSF, USDA, etc.) and evidence of approval

☐ Nongovernmental agency (e.g., University review, Other specify):

☐ Departmental Review: I assure that this project has been reviewed and approved for scientific or instructional merit by:

☐ Expert reviewer (Name)

☐ Departmental Committee Review (Committee Name and Chairperson):

☐ Other (Describe):

Signature: _____ 01/31/2014

Department Head Date

XII. REVIEWED AND APPROVED BY IACUC REVIEWERS

Signature: _____ 01/31/2014

Institutional Animal Care and Use Committee Chair Date

Signature: _____ 01/31/2014

Institutional Animal Care and Use Officer Date

Following action on this application, copies of approval or denial letters will be sent to the applicant, Department Head, and appropriate College Dean who will also receive a copy of this application.

APPENDIX B

Exp 1 Nicotine Nonpreferred

Saline	Nicotine	NTX_Saline
417	386	271
179	280	170
300	152	312
314	322	246
275	348	241
415	231	167
338	300	339
201	89	283

Exp 1 Nicotine Change

Before	After
232	386
248	280
231	152
229	322
255	348
141	231
240	300
214	89

Exp 1 Nicotine- Saline percent changed to nonpreferred

Saline	Nicotine	ntx_saline
184.5133	166.3793	169.0229
77.15517	112.7517	86.88245
153.8462	65.80087	118.3312
251.8717	140.6114	102.3578
128.5047	136.4706	106.3235
169.6185	164.218	84.48566
183.3635	125.1739	155.2672
80.07968	41.65367	113.5027

Exp 1 Naltrexone Nonpreferred

Saline	Ntx 1.0	Ntx 3.0
271	210	341
170	66	70
312	246	154
246	149	304
241	365	232
167	209	134
339	300	374
283	325	106

Exp 1 Naltrexone Change 1.0

Before	After
294	210
245	66
151	246
234	149
288	365
268	209
182	300
284	325

Exp 1 Naltrexone Change 3.0

Before	After
282	341
250	70
268	154
153	304
236	232
229	134
288	374
182	106

Exp 1 Naltrexone - Saline percent changed nonpreferred

Saline	Ntx 1.0	Ntx 3.0
169.0229	71.42857	120.7792
86.88245	26.90217	27.96272
118.3312	162.9139	57.53425
102.3578	63.67521	198.6928
106.3235	126.883	98.16644
84.48566	77.98508	58.51529
155.2672	165.1376	129.8611
113.5027	114.3025	58.24176

Exp 2 Nicotine Nonpreferred

Nicotine	Saline
218	233
302	201
253	126
328	229
255	227
339	216
249	180
280	304
185	162
301	126

Exp 2 Nicotine Change

Before	After
199	218
279	302
89	253
251	328
189	255
231	339
282	249
205	280
163	185
277	301

Exp 3 Nicotine + Naltrexone Nonpreferred

Saline + Saline	Naltrexone + Saline	Saline + Nicotine	Naltrexone + Nicotine
102	366	205	232
211	221	310	190
113	142	253	200
256	275	244	263
281	133	251	132
262	245	302	142
367	167	386	199
198	199	406	170
49	134	231	394
257	455	211	106

Exp 3 Nicotine + Naltrexone Change

Saline + Saline	Naltrexone + Saline	Saline + Nicotine	Naltrexone + Nicotine
-129	140	-34	-29
-48	-64	107	-86
-97	-11	-29	-16
-36	-15	-17	34
47	-139	-12	-101
15	-1	15	-131
76	-57	110	-27
-55	-80	130	-97
-145	-133	-50	134
10		-30	-128

Exp 3 Nicotine + Naltrexone Two Factor Before After

	Saline + Saline									
	A:Y1	**A:Y2**	**A:Y3**	**A:Y4**	**A:Y5**	**A:Y6**	**A:Y7**	**A:Y8**	**A:Y9**	**A:Y10**
Before	102	211	113	256	281	262	367	198	49	257
After	231	259	210	292	234	247	291	253	194	247

	Naltrexone + Saline									
	B:Y1	**B:Y2**	**B:Y3**	**B:Y4**	**B:Y5**	**B:Y6**	**B:Y7**	**B:Y8**	**B:Y9**	**B:Y10**
Before	366	221	142	275	133	245	167	199	134	
After	226	285	153	290	272	246	224	279	267	

	Saline + Nicotine									
	C:Y1	**C:Y2**	**C:Y3**	**C:Y4**	**C:Y5**	**C:Y6**	**C:Y7**	**C:Y8**	**C:Y9**	**C:Y10**
Before	205	310	253	244	251	302	386	406	231	211
After	239	203	282	261	263	287	276	276	281	241

	Naltrexone + Nicotine									
	D:Y1	**D:Y2**	**D:Y3**	**D:Y4**	**D:Y5**	**D:Y6**	**D:Y7**	**D:Y8**	**D:Y9**	**D:Y10**
Before	232	190	200	263	132	142	199	170	394	106
After	261	276	216	229	233	273	226	267	260	234