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Novel Therapy for Nicotine Addiction in Alcohol Dependent Rats

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NOVEL THERAPY FOR NICOTINE ADDICITION IN ALCOHOL DEPENDENT RATS

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Abstract

The co-dependence of nicotine and alcohol addiction occurs at high rates, complicates treatment, and is often associated with significant morbidity and mortality. Treatment options of alcohol and tobacco co-dependence are limited. Currently, there are drugs available for nicotine dependence or alcohol dependence. However, there are no therapeutic drugs available on the market for the co-dependence of nicotine and alcohol. Therefore, and important opportunity of new therapeutic options and drug development has presented itself. NT69L, a non-selective neurotensin (NT) agonist, provides a potential novel therapy for nicotine addiction in alcoholics by interacting with the common neurotransmitter circuits supporting the rewarding process for both nicotine and alcohol. Considering the behavioral effects of NT69L in attenuating nicotine self-administration in rats and alcohol consumption in mice, the present study was designed to assess the effects of NT69L as a new drug. NT69L was used in the treatment of nicotine addiction in an animal model of alcoholics and in attempts to attenuate withdrawal signs associated with nicotine and alcohol dependence. Wistar rats pre-exposed to alcohol vapor or air were allowed to self-infuse nicotine (0.03mg/kg/infusion) or saline. When the rats reached a stable level of responding, the effect of pretreatment with NT69L (1mg/kg i.p.) on the reinforcing effect of nicotine was determined. Animals self-infused nicotine at a significantly ($p < .05$) higher rate compared to saline in both air and alcohol vapor exposed groups. Acute pretreatment with a single injection of NT69L significantly ($p < .05$) reduced nicotine self-infusion in both the alcohol vapor and the air exposed groups for 5 days post-injection. Additionally, NT69L attenuated the alcohol- and nicotine-induced withdrawal signs associated with the discontinuation of alcohol and nicotine administration. Neurotensin agonist, NT69L, may represent a potential novel therapy to treat the co-addiction of alcohol and nicotine.

Novel Therapy for Nicotine Addiction in Alcohol Dependent Rats

The three most commonly used non-therapeutic drugs in the Western societies are caffeine, nicotine, and ethanol (Rang, Dale, Ritter, & Moore, 2003). The combination of nicotine and alcohol addiction frequently occurs in humans (DiFranza & Guerrera, 1990). Drug addiction is typically described as a chronically relapsing disorder involving repeated periods of compulsive drug seeking and use which continues despite potential adverse consequences associated with the behavior (Koob & LeMoal, 1997). Unfortunately, drug addiction is a common disorder and the extent of worldwide drug use is estimated to include 2 billion alcohol users, 1.3 billion smokers and 185 million illicit drug users (World Health Organisation [WHO], 2002). Addiction is increasingly clinically recognized as a neurobiological disease. It is believed that addiction's manifestation and enduring nature is influenced by a combination of behavioral, genetic and psychosocial factors (Jupp & Lawrence, 2010).

Addictive substances influence an individual to continue taking the drug by acting as a stimulus-reward learning paradigm which leads to the acquisition of drug-reinforced habits over time (Cador, Robbins, & Everitt, 1989). Dependence-producing drugs possess the common feature of having a positive reinforcing action (reward) associated with activation of the reward pathway. The reward pathway includes the mesolimbic dopaminergic pathway which runs via the medial forebrain bundle, from the ventral tegmental area of the midbrain to the nucleus accumbens and limbic region. Activation of this pathways results in a hedonic, or pleasurable, effect (Rang et al., 2003).

In combination with the hedonic effect of the drug, there is usually a process of habituation, or adaptation, when the drug is given repeatedly or continuously. This adaptation

results in a condition in which the cessation of the drug has an aversive effect on the individual. Drug dependence describes the state when the taking of a drug becomes compulsive and maintains priority over other needs. Withdrawal syndrome describes the unpleasant effects, both physical and psychological, from the cessation of drug use. Each type of drug varies in the intensity and nature of the physical withdrawal syndrome. The major factor leading to the relapse in drug addicts is the psychological dependence, which usually outlasts the physical withdrawal syndrome (Rang et al., 2003).

Nicotine Use and Dependence in Humans

Smoking remains one of the leading causes of preventable death in the world (Jupp & Lawrence, 2010). One out of every five deaths in the United States every year is related to smoking which equates to about 1,200 deaths each day. A person's life is thought to be shortened by fourteen minutes each time a cigarette is smoked. Putting this statement into a long-term perspective, smoking two packs a day for twenty years can reduce a person's lifespan by approximately eight years (Levinthal, 2010). The world-wide prevalence of smoking is now about 18% of the adult population, each smoker uses on average 5,000 cigarettes per year (Rang et al., 2003).

Nicotine is a psychomotor stimulant that has a very strong dependence liability (Rang et al., 2003). Nicotine stimulates a release of dopamine in the nucleus accumbens. The nucleus accumbens is the same area of the brain responsible for the reinforcing properties of other drugs such as alcohol, cocaine, and opiates (Pontieri, Tanda, Orzi, & DiChiara, 1996). At a cellular level, nicotine acts on nicotinic acetylcholine receptors to cause neuronal excitation and the central effects of nicotine can be blocked by the use of receptor antagonists (Rang et al., 2003).

Physical Withdrawal from Nicotine

Regular nicotine use leads to tolerance, physical dependence, and psychological dependence (craving). Most smokers would like to quit, but few succeed. Difficulties in giving up the smoking habit are frequently reported to be related to the nicotine withdrawal syndrome (Snyder, Davis, & Henningfield, 1989). Symptoms of the nicotine withdrawal syndrome are associated with strong urges to smoke during attempts to quit (Doherty, Kinnunen, Militello, & Garvey, 1995). A physical withdrawal syndrome is known to occur in both humans and experimental animals accustomed to regular nicotine administration. The injection of nicotine in rats is a frequent method used to mimic the acute effects of smoking (Rang et al., 2003).

The nicotine withdrawal syndrome includes increase nervousness, frustration, anger, and desire to smoke (Snyder et al., 1989). Symptoms begin within roughly six hours after the last cigarette. First, a smoker's heart rate and blood pressure will decrease. Over the next twenty-four hours, common symptoms that occur include headache, inability to concentrate, irritability, drowsiness, fatigue, insomnia, as well as other sleep disturbances (Kolslowski et al., 1989). In a study of people who smoked and were simultaneously in some form of other drug abuse treatment, 74% judged the difficulty of quitting smoking to be at the same level if not more difficult than stopping their drug of treatment focus. In the same study, one in three individuals considered it "much harder" to quit smoking (Levinthal, 2010).

Pharmacotherapeutic Approaches to the Treatment of Nicotine Addiction

Several pharmacotherapeutic options have been approved by the United States Food and Drug Administration (USFDA) for the treatment of nicotine addiction. Nicotine replacement therapy is the main pharmacological approach used for treatment. A variety of these products are available and differ based on the route of nicotine administration which include the transdermal patch, gum, inhaler, nasal spray, lozenge, and microtab (Jupp & Lawrence, 2010). The two most

common nicotine replacement therapies chosen are the chewing gum form, used several times daily, or a transdermal patch, which is replaced daily. These methods cause various side effects such as nausea, gastrointestinal cramps, coughing, insomnia, and muscle pains. Transdermal patches often cause irritation and itching on the portion of the skin where the patch was applied (Rang et al., 2003).

Despite various evidence found on the efficacy of these pharmacotherapies between six and twelve months following cessation, long term follow-up studies (twelve months or greater) have found the rate of continued abstinence to be low (Eisenberg et al., 2008). Attempts at long-term cessation succeed in only about 20% of the cases (Rang et al., 2003).

Alcohol Use, Abuse, and Dependence in Humans

Alcohol is a general central nervous system depressant with a strong dependence liability (Rang et al., 2003). Studies have shown that alcohol dependence is related to neural activity in the same dopamine-releasing receptors in the nucleus accumbens that have been implemented in craving for heroin, cocaine, and nicotine (Levinthal, 2010). Alcohol dependence criteria is described by the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev.; *DSM-IV-TR*; American Psychiatric Association, 2000) and can be classified as a syndrome in which alcohol abuse involves a variety of significant physical, psychological, social, and behavioral problems. Based on data averaged from surveys in 2002, 2003, and 2004, the number of Americans who met the criteria for alcohol abuse or dependence in the past year is 18.2 million (Levinthal, 2010). Approximately 8.5% of adults in the United States (about one in twelve), are either alcohol abusers or alcohol dependent according to the DSM-IV-TR standards.

Alcohol abuse is a syndrome primarily characterized by the continuous use of alcohol despite the drinker’s knowledge of having a persistent or reoccurring negative physical reaction

or some difficulty in social or occupational functioning. The highest percentages of "heavy drinkers" are found in people between the ages of 18 to 25 years. The "heavy drinker" terminology is categorized as having consumed five or more alcoholic drinks during the same occasion on at least five different days within the previous month (Grant et al., 2004). Neurological degeneration occurs in heavy drinkers, causing dementia and peripheral neuropathies. Long-term alcohol consumption causes liver disease, progressing to cirrhosis and liver failure. In contrast, moderate alcohol consumption has a protective effect against ischemic heart disease (Rang et al., 2003).

Physical Withdrawal from Alcohol

Physical withdrawal symptoms from alcohol are classified in two clusters. One cluster, termed the alcohol withdrawal syndrome, is the more common of the two clusters. It begins with insomnia, vivid dreaming, and a severe hangover. These symptoms are followed by tremors ("the shakes"), sweating, mild agitation, anxiety (the "jitters"), nausea, vomiting, increased heart rate, and increased blood pressure. As the central nervous system rebounds from the chronic depression induced by alcohol, there are also brief tonic-clonic (grand mal) seizures in some patients. The alcohol withdrawal syndrome usually reaches a peak from twenty-four to thirty-six hours after the last drink and tends to be over after forty-eight hours. A similar syndrome of central and autonomic hyperactivity can be produced in experimental animals by ethanol withdrawal (Rang et al., 2003). The second cluster, called delirium tremens, is less common but much more dangerous. The symptoms include extreme disorientation, confusion, profuse sweating, fever, disturbing nightmares, and periods of frightening hallucinations. These effects generally reach a peak three to four days after the last drink. The possibility of life threatening events such as heart failure, dehydration, or suicide is present during this time (Levinthal, 2010).

Pharmacotherapeutic Approaches to the Treatment of Alcoholism

Alcohol dependence, also knows as alcoholism, is present in 4-5% of the population and as with smoking, very difficult to effectively treat. The main pharmacological approaches used are as follows: (1) to alleviate the acute abstinence syndrome during withdrawal (benzodiazepines); (2) to render alcohol consumption unpleasant (disulfiram); (3) to reduce the reward of alcohol intake (naltrexone); and (4) to reduce craving (acamprosate) (Rang et al., 2003).

Historically, to prevent the relapse to alcohol consumption, disulfiram was the first medication used. Disulfiram took effect by the way of aversion therapy (Kitson, 1977). Disulfiram (brand name: Antabuse) is a medication that when combined with alcohol, causes severe physical reactions and discomfort (Levinthal, 2010). Clinical trials employing disulfiram have produced mixed results, with high rates of noncompliance and risk of toxicity reported (Ehrenreich & Krampe, 2004).

Currently, other United States Food and Drug Administration (USFDA) approved therapeutics have been shown to be somewhat effective for the treatment of alcoholism. These therapeutics include the non-selective, long-lasting opioid receptor antagonists, naltrexone (brand name: ReVia) and nalmefene (brand name: Revex) (Jupp & Lawrence, 2010). Acamprosate (brand name: Campral) is a GABA-related drug for the treatment of alcoholism (Levinthal, 2010). Acamprosate is a synthetic GABA analogue that is thought to reduce alcohol consumption via several potential mechanisms. Naltrexone is one of the most commonly prescribed pharmacotherapies for encouragement of abstinence in alcoholics (Jupp & Lawrence, 2010).

Despite the availability of multiple different therapeutic options for the treatment of alcoholism and the demonstrated utility they offer, the treatment effect size remains small. Naltrexone, nalmefene, and disulfiram are limited by rates of noncompliance. Acamprosate has shown a development of tolerance to the effect of the drug itself. Considerable attempts have been dedicated to determining how to boost the efficacy of these treatments. Efforts have also focused on the development and investigation of new therapeutics with alternate mechanisms of action for the treatment of alcohol dependence (Jupp & Lawrence, 2010).

Combination use of Nicotine and Alcohol in Humans

Cigarette smoking is highly prevalent among people with alcohol use disorders such as alcohol abuse and dependence. Smoking rates in treatment-seeking populations may be as high as 80% (Hughes, 1996). Clinical and epidemiological studies approximate that 50 to 80% of alcohol-dependent individuals are regular smokers and have higher rates of nicotine dependence (Romberger & Grant, 2004). Current alcohol use and abuse problems are also coupled with higher levels of nicotine dependence and a lower probability of smoking cessation (Breslau, Peterson, Schultz, Andreski, & Chilcoat, 1996). Cravings for nicotine have been reported as higher in alcohol-dependent smokers than in non alcohol-dependent smokers (Hertling et al., 2005). Alcohol consumption is also significantly higher in individuals who smoke as compared with alcohol only users (York & Hirsch, 1995).

In general, humans that co-abuse alcohol and nicotine have worse clinical outcomes than individuals who use only alcohol or nicotine alone (Lajtha & Serchen, 2010). Comorbid cigarette smoking and alcoholism has been associated with higher rates of depression compared with nonalcoholic smokers (Marks, Hill, Pomerleau, Mudd, & Blow, 1997). The combination of nicotine and alcohol addiction complicates treatment and is frequently associated with significant

morbidity and mortality (Daeppen et al., 2000; Zacny, 1990). Additionally, alcohol-dependent smokers are more likely to die from smoking related diseases rather than directly from an alcohol related medical condition. Given the increased tobacco-related mortality and morbidity in alcohol-dependent smokers (Hurt et al., 1996), and the greater difficulty in quitting smoking, it is critical to identify the most effective treatment possible.

Self-administration as a Drug Dependence Model in Rats

The core dimension of drug dependence can be modeled in animals by means of drug self-administration methods. Voluntary self-administration behaviors in laboratory animals have been supported by drugs that are commonly abused by humans (Griffiths, Bigelow, & Henningfield, 1980). To study the positive reinforcing effect of drugs, there are many choices of animal species and operant paradigms to use. It was demonstrated by Corrigall (1999) that animals will do work, sometimes substantial amounts, to obtain nicotine. One particular paradigm of interest is a model in which a task is performed by a laboratory animal, such as pressing a lever, to obtain intravenous infusions (self-administration) of nicotine (Corrigall, 1999).

Corrigall and Coen (1989b) developed reliable schedules of intravenous nicotine self-administration in rats. Rats acquire stable self-administration under specific methodological conditions. Important schedule parameters include: (1) a short infusion time of 1-2 seconds for nicotine; (2) limitation of aversive effects due to nicotine overdosing (e.g. limited daily access to a self-administration session, a "timeout" period after each infusion, and control of nicotine solutions pH); and (3) a fixed-ratio schedule between responding and nicotine infusion delivery (Corrigall & Coen, 1989a). Another important procedure is diet restriction during self-administration studies. Diet restriction allows for control of rat body weight, which is an

important factor in order to hold infusion volumes constant (infusion unit, mL/kg of body weight) over every experimental session (Corrigall & Coen, 1989a; Singer, Simpson, & Lang, 1978).

There are several reasons as to why we model nicotine dependence in laboratory animals. One reason is due to the clear parallels between drug-taking by humans and drug self-administration studies in animals (Griffiths et al., 1980). Of particular interest is the similarity between self-administration (the intravenous route) of nicotine by animals, and by the inhalation of tobacco smoke by humans (Rose & Corrigall, 1997). The intravenous route mimics the rapid onset of nicotine delivered by the inhalation of tobacco smoke in humans and allows for quantifiable doses of nicotine to be delivered. This similarity points to the clear utility of animal models in the preclinical testing of pharmacotherapeutic options for the treatment of nicotine addiction. Ways in which animal models of nicotine reinforcement have contributed to the development of potential treatments have been to aid in the discontinuation of nicotine use, and to the determination of the possibility of dependence to such pharmacotherapeutic options themselves (Corrigall, 1999).

Another reason why animal models of nicotine dependence have been used is the allowance for the investigation of the biological mechanisms by which drugs control behavior. Research on nicotine dependence models has contributed significant information to our understanding of the neuronal and neurochemical elements that are involved in drug abuse (Corrigall, 1999; Corrigall & Coen, 1989b). Therefore, this information has lead to the developmental foundation of preclinical pharmacotherapeutic options for the treatment of nicotine dependence.

Withdrawal Symptoms in Rats

Nicotine. A discontinuation of chronic nicotine administration in rats results in the appearance of a withdrawal syndrome characterized by several somatic signs such as eye-blinks, body-shakes, gasps and abdominal writhes (i.e. "somatic signs of nicotine withdrawal"). Similar signs have been reported in human smokers (Hughes, 1996). Therefore, the appearance of the somatic nicotine withdrawal signs in rats reflects the aspects of nicotine withdrawal corresponding to those observed in human smokers.

Alcohol. According to Gilpin et al. (2009), alcohol dependence in rats can be identified by the presence of physical and affective disturbances brought on by the absence of alcohol. Physical symptoms of alcohol dependence become apparent during acute withdrawal and subside within 24-48 hours following the discontinuation of alcohol exposure. The physical symptoms include convulsions, motor abnormalities, and autonomic disturbances (Gilpin et al., 2009). In order of ascending severity, somatic withdrawal symptoms in rats include: general hyperactivity, tail spasticity/tremors, general spasticity/tremors, head tremors, wet shakes, teeth chattering, and spontaneous tonic-clonic convulsions (Majchrowicz, 1975).

Neurotensin (NT)

Neurotensin is a neuropeptide that is closely associated with, and modulates dopamine (Bissette & Nemeroff, 1988), acetylcholine, glutamate, and GABA neurotransmission implicated in addiction and reward pathways. Local administration of neurotensin in the prefrontal cortex (PFC) increases extracellular levels of acetylcholine and GABA (Petkova-Kirova et al., 2008). In support of these findings are results with the neurotensin agonist NT69L (Prus, Huang, Li, & Meltzer, 2007), which was developed in the Neuropsychopharmacology laboratory at Mayo Clinic. Neurotensin has also been reported to enhance GABAergic activity in rat hippocampus (Li, Geiger, & Lei, 2008) and to reduce glutamatergic neurotransmission in dorsolateral striatum

(Yin, Adermark, & Lovinger, 2008). However, neurotensin must be administered centrally to have an effect because it is easily degraded by peptides in the digestive system. The Neurophsychopharmacology laboratory has developed a number of neurotensin agonists that can be administered systemically and maintain the central effects of neurotensin. The most studied of these agonists is NT69L which binds with equal affinity to the two well characterized neurotensin receptors (NTS1 and NTS2).

Neurotensin and Addiction

Research with NT69L shows that it blocks nicotine-induced sensitization and nicotine self-administration (Boules et al., 2011; Fredrickson, Boules, Yerbury, & Richelson, 2003a; Fredrickson, Boules, Yerbury, & Richelson, 2003b). In addition to its role in animal models for nicotine addiction, the neurotensin system has been strongly implicated in the neurochemical and behavioral effects of alcohol use (Ehlers et al., 1999; Erwin, Campbell, Myers, & Womer, 1995). Chronic administration of NT69L reduces alcohol preference and consumption in mice through modulation of the neurotensin receptor subtype 1 (NTS1) (Lee et al., 2010). Biochemically, NT69L normalizes the nicotine-induced changes in dopamine (Liang et al., 2008), the neurotransmitter that promotes the motivational process for both nicotine and alcohol, and the alcohol-induced increase in dopamine and glutamate in the striatum (Li, Boules, & Richelson, 2011). Additionally, neurotensin modulates other neurotransmitters implicated in alcohol use disorder. It causes neurochemical changes similar to acamprosate (the calcium salt of acetylhomotaurine), which is one of the FDA-approved drugs to treat alcohol use disorder. Administration of acamprosate or NT69L increases extracellular concentration of dopamine in striatum (Li et al., 2008; Prus et al., 2007) and both acamprosate and NT69L modulate glutamate (Dahchour & De Witte, 2000).

Pharmacotherapeutic options for the treatment of alcohol and tobacco co-dependence are limited. Thus, there is a critical need for the development of novel drugs implementing new therapeutic targets. Considering the behavioral effects of NT69L in attenuating nicotine self-administration in rats and alcohol consumption in mice, and its modulatory effects on neurotransmitters implicated in nicotine and alcohol addiction, this study was set to test the efficacy of NT69L as a potential novel therapy for nicotine addiction in alcohol dependent rats. In this study, we hypothesize that pre-treatment with neurotensin agonist, NT69L, will (1) attenuate nicotine self-administration in alcohol dependent rats, and (2) will decrease alcohol and nicotine withdrawal signs.

Materials and Methods

Experimental Animals

Male Wistar rats (Harlan, Indianapolis, IN, USA) weighing 200-220g at the beginning of the experiment were tested. Animals were housed in temperature controlled rooms with free access to food and water unless otherwise indicated. Lights were on a 12 hour light/dark cycle with lights on at 6:00 AM. All animal procedures were approved by Mayo Clinic and University of North Florida Institutional Animal Care and Use Committees.

Behavioral Studies

Operant Behavior

Apparatus. Six operant conditioning chambers (Med Associates, St Albans, VT, USA) were required for this study. The chambers were placed in sound-attenuated outer chambers in order to eliminate outside noise pollution. The two sidewalls of the chambers were aluminum with the front and back walls made of clear Plexiglas. On the right side-wall of the chamber, two

response levers were located on either side of a food trough. A 28-V DC white cue light was located above each of the response levers. Mounted about each chamber was an infusion pump (Med Associates), which was used to deliver the nicotine or saline into the animal's jugular vein via a tether and swivel line assembly (Instech Labs, Inc. PA, USA). The swivel allowed unrestricted movement of the rats throughout the chamber during the sessions.

Operant training

One week after arrival, the rats were placed on a restricted food intake in order to reduce their body weight to 85% of their *ad libitum* weights. Rats were then trained to self-administer sucrose pellets orally in daily one hour sessions to facilitate the learning process of the operant response. The rats were trained on a continuous reinforcement of a fixed ratio 1 schedule (FR1). Each response resulted in the delivery of one sucrose pellet with a maximum of 20 reinforcements per session. Reinforcement was paired with a 20 second cue light, during which the animal was reinforced upon pressing the active (right) lever. The cue light was followed by a 60 second period during which the cue light was turned off and responding was not reinforced. Responding to the inactive (left) lever had no rewards or consequences. Responses for both active and inactive levers and infusions were recorded by Med Associates computer software. During the operant learning and infusion phases of the experiment, food access was restricted to 20 g/rat/day given immediately after each operant session (Boules et al., 2011). After a stable level of responding was achieved, rats were allowed free access to food and water prior to undergoing surgery to insert a jugular catheter as described below.

Alcohol Dependence

Rats were exposed to ethanol vapor on a schedule of 14 hours on/10 hours off rotation for three weeks to induce alcohol dependency as described in detail by others (Gilpin, Richardson,

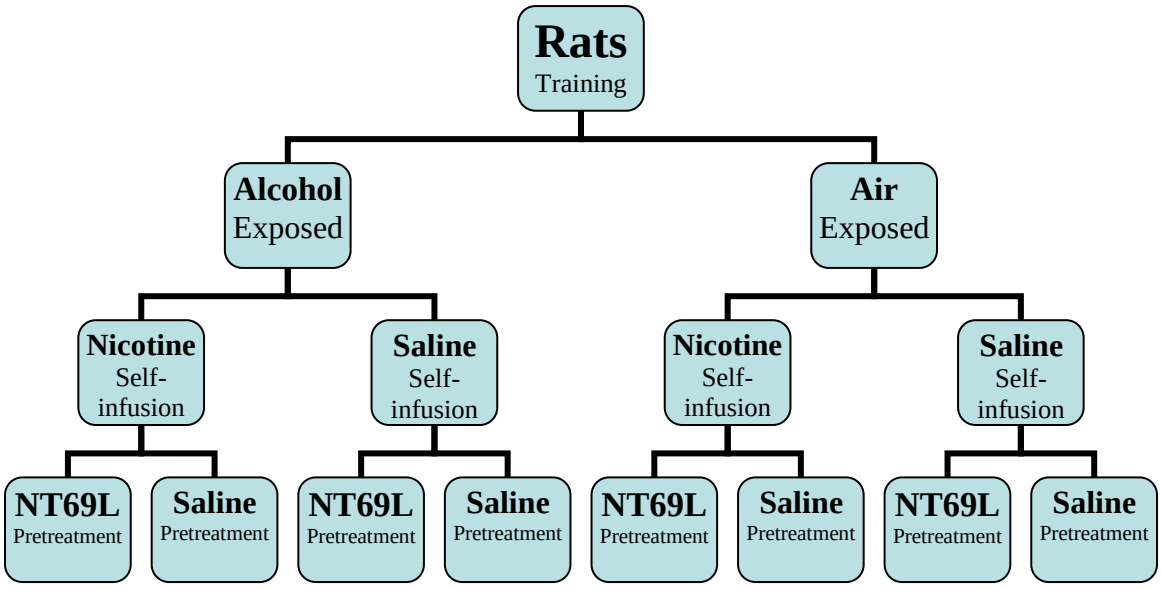
Lumeng, & Koob, 2008; Gilpin, Richardson, Cole, & Koob, 2008). Briefly, the rats were placed (three to a cage) in two sealed Plexiglas standard rodent cages (Alcohol Research Inc, La Jolla San Diego, CA, USA). The chambers were connected to a timer that turned the ethanol vapor on and off every day. Ethanol vapor was produced by dripping 95% ethanol into a 2000 ml Erlenmeyer vacuum flask kept on a warming tray (50C). Air was blown over the bottom of the flask (at a rate of 2.268 l/min and then into the rat cages to vaporize the ethanol (O'Dell, Roberts, Smith, & Koob, 2004).

Measurement of blood alcohol levels (BALs). Blood alcohol levels (BAL) were determined at the end of the 14 hour ethanol vapor exposure period. Blood samples were collected and centrifuged to separate the plasma serum from blood. The plasma (5 µl) was extracted and then injected into an Analox AM1 analyzer for measurement (Analox Instruments LTD, Lunenberg, MA). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O_2→ acetaldehyde + H_2O_2). The rate of oxygen consumption is directly proportional to the alcohol concentration (Gilpin et al., 2008). Control rats were treated equally except they were exposed to air.

Nicotine self-infusion

Self-infusion of nicotine was assessed and recorded during daily 1 hour sessions. Two groups of rats, one group of rats pre-exposed to alcohol vapor and one control group (pre-exposed to air), were allowed to self-infuse nicotine (0.03 mg/kg/infusion in 90 µl volume) or saline contingent upon pressing the active lever (right). The lever press triggered an IV dose from the syringe pump (Med Associates). The active lever is the same lever that was used for both nicotine/saline infusion and for sucrose pellet reinforcement. When the animals reached a stable level of responding, the rats in each group were further subdivided into 2 groups. One

group was pretreated with NT69L (1 mg/kg i.p.) and the other with an equal volume of saline 30 minutes before the nicotine/saline self-infusion session (Boules, et al., 2011). The rats continued to be tested daily for five days post-NT69L injection. The dose of nicotine used in this study has been reported by several investigators to be at or near the peak of the dose-response curve for nicotine self-administration (Corrigall & Coen, 1989b; Corrigall, Franklin, Coen, & Clark, 1992). The dose of NT69L was that which has been previously used in our laboratory to block initiation and expression of nicotine-induced locomotor sensitization in rats, a mechanism that is thought to underlie craving and risk of relapse to smoking (Fredrickson et al., 2003a, Fredrickson et al., 2003b, Fredrickson, Boules, Lin, & Richelson, 2005). The diagram below shows a pictographic representation of the division of rats among experimental conditions.



Withdrawal Studies

Nicotine Withdrawal

Two groups of Wistar rats (n=12) were exposed to either alcohol vapor (14h on/ 10h off) or air for 3 weeks. Animals were then injected with nicotine (0.35 mg/kg s.c.) twice daily for 15 days. On day 15, nicotine injections were discontinued and the animals were instead injected with either NT69L (1 mg/kg i.p.) or saline. Thirty minutes later, somatic nicotine withdrawal signs were recorded over a 10 minute observation period. Somatic withdrawal signs recorded include body shakes, chews, cheek tremors, eye blinks, foot licks, genital licks, head shakes, teeth chattering, and writhes as described by Skjei and Markou, (2003). The withdrawal signs were recorded as the sum of each occurrence of the withdrawal signs during the 10 minute period. Observations of withdrawal signs were taken prior to nicotine exposure (baseline), after 14 days of nicotine injections, and after NT69L/saline injections (day 15).

Alcohol Withdrawal

Two groups of Wistar rats were exposed to either alcohol vapor (14h on/ 10h off) or air for three weeks. Acute alcohol withdrawal was achieved by the discontinuation of the alcohol vapor exposure. Rats were tested for alcohol withdrawal behavior two hours after anticipated exposure to alcohol vapor. Alcohol withdrawal behavior included observation for tail rigidity (Gilpin et al., 2008), body tremors, and wet dog shakes (Uzbay & Kayaalp, 1995). Alcohol withdrawal tests included assessment of hyperactivity, muscle rigidity (catatonia) (Uzbay & Kayaalp, 1995), hyperalgesia (excessive sensitivity to pain) (Gatch & Lal, 1999), and hypothermia. Measures of alcohol withdrawal were taken prior to alcohol exposure (baseline), after 3 weeks of alcohol exposure, and after NT69L (1 mg/kg i.p.) or saline injections.

Hyperlocomotion. Locomotor activity was recorded in a Plexiglas Opto-Varimex activity chamber (Columbus OH, USA) equipped with infrared photocell emitters and detectors. The rats were allowed to acclimate in the room for 1 hour and then placed in the activity chambers. Activity was recorded as the distance traveled in centimeters every 10 minutes within a 2 hour period.

Hyperalgesia. During alcohol withdrawal, hyperalgesia (excessive sensitivity to pain) was recorded with the use of an Analgesia Meter (Harvard Apparatus, Holliston, MA, USA), which assesses the tail flick latency to radiant heat. The latency for the rat to flick its tail was measured in seconds. A cutoff time of 15 seconds was implemented to prevent tissue damage.

Muscle rigidity (catatonia). Muscle rigidity was evaluated with the use of the bar test. The rats' front paws were placed on a suspended metal bar 10 mm in diameter and 11 cm from the base of a plastic standard rat cage. The latency for the rat to remove its paws from the bar was recorded in seconds. Each rat was tested three times and the average time was recorded.

Hyperthermia. Body temperature was measured by means of a thermistor probe inserted approximately 2 cm into the rectum of the rat. Baseline temperature readings were recorded prior to alcohol exposure. Body temperature was recorded again after the rats were exposed to either alcohol or air for three weeks and third time 30 minutes post NT69L injection. The data were recorded and displayed as the change in body temperature from baseline in ºC for each rat. The average change in temperature of all rats in each group was illustrated.

Surgical procedures

Jugular catheterization

Animals underwent surgery to place an indwelling catheter for nicotine self-infusion as done previously (Boules et al., 2011; Corrigal, 1999) with slight modifications. Briefly, the rats

were anesthetized by an intramuscular injection of ketamine (30 mg/kg), xylazine (6 mg/kg), and acepromazine (1 mg/kg) cocktail. A silastic catheter was inserted into the jugular vein and exited and secured between the scapulae with the use of vascular access button and covered with protective cap (Instech Labs, Inc. PA, USA). The vascular access button permits quick, aseptic connection and disconnection of the catheterized rat to the infusion tether. A protective cap allows for group housing without the possibility of damaging the vascular access buttons and catheters. For pain management, 100-300 mg/kg of 30 ml Children's Tylenol (160 mg/5 ml formulation) was added to drinking water 48 hours before and 48 hours after surgery. A daily injection of ampicillin (100 mg/kg) was given for 3 days post-surgery. The animals were allowed a one week recovery period prior to returning to operant testing.

Drugs and Compounds

NT69L

NT69L, a neurotensin (NT) agonist, was synthesized by Mayo Clinic Chemistry Core facility as described previously (Fauq et al., 1998). NT69L is modifies from NT at amino acid positions located in the C-terminal 8-13 sequence. Table 1 shows the structure of NT69L as compared to neruotensin.

Table 1: Structures of NT and NT69L

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
NT	*p*-Glu	l-Leu	l-Tyr	l-Glu	l-Asn	l-Lys	l-Pro	l-Arg	l-Arg	l-Pro	l-Tyr	l-Ile	l-Leu
NT69L	–	–	–	–	–	–	–	*N*-methyl-Arg	l-Lys	l-Pro	l-neo-Trp	Tert-Leu	l-Leu

Nicotine

Nicotine hydrogen tartrate salt was purchased from Sigma Chemical Co., (St Louis, Mo., USA) and dissolved in sterile physiological saline (0.9% sodium chloride). For nicotine self-

infusion, after being dissolved into saline, the pH was adjusted to 7 (±0.5) and then filtered through a 0.2 μm filter.

Alcohol

Ethanol (190 proof/ 95%) used for the vapor chambers was purchased from Fisher Thermo Scientific Inc., (Houston, TX, USA).

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data. Individual group comparisons were carried out using the Tukey's test with the use of SigmaStat 2.0 software (SPSS, Inc., Chicago, IL), with $p < .05$ being considered significant.

Results

Behavioral Studies

Operant Behavior

Effect of NT69L on nicotine self-administration in rats pre-exposed to alcohol vapor or air. The one way ANOVA shows a significant effect of treatment on nicotine self-administration, $F(7, 50) = 25.293$, $p < .001$.

Nicotine self-administration was significantly higher than saline self-administration in both the alcohol vapor and air exposed groups ($p < .001$). Pre-exposure to alcohol significantly decreased nicotine self-administration ($p < .001$). Pretreatment with NT69L 30 minutes prior to nicotine self-administration session significantly reduced nicotine self-administration in both the alcohol vapor and air exposed groups ($p < .005$). This finding is shown in Figure 1. Given that all the rats were trained to press the lever for sucrose pellets, the lack of response in the saline-infusion group established that the responses in the nicotine-infusion group were specific to

nicotine. This report also confirmed that the responding to the sucrose pellets had been extinguished and was not carried over to compromise responding to nicotine.

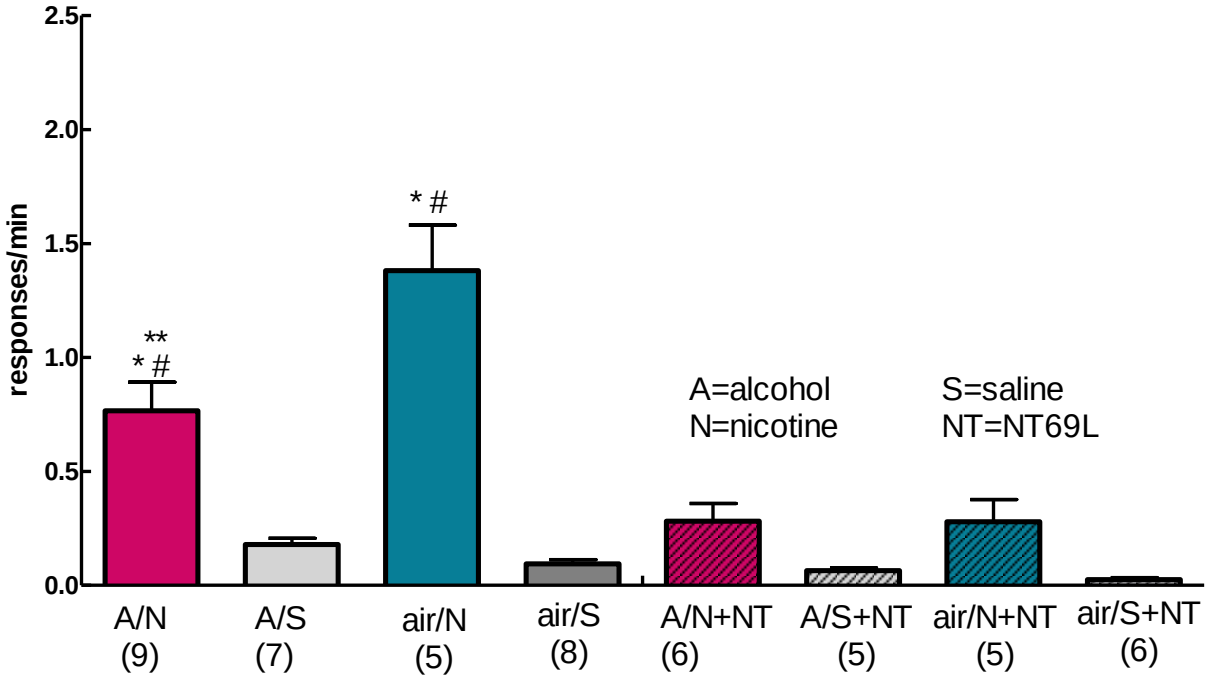


Figure 1

Effect of NT69L on nicotine self-infusion in alcohol- and air-exposed Wistar rats.
NT69L (1mg/kg i.p.) or saline was injected 30 min prior to nicotine self-infusion (0.03 mg/kg/infusion in 90 µl sterile saline) session on FR1 schedule of reinforcement. Values represent the number of active nicotine self-infusions (mean ± SEM); (n) number of animals in each group; * significantly different from respective saline groups ($p < .001$); ** significantly different from air/N group ($p < .001$); # significantly different from corresponding NT69L pretreated groups ($p < .005$).

Figure 2 shows the time course indicated by each consecutive session of nicotine self-administration after the responding to nicotine self-infusion had stabilized. The attenuating effect of NT69L on nicotine self-administration lasted for 5 days post-injection. Previous studies (Boules et al., 2011) showed that NT69L has no reinforcing effect and that there was no significant difference between NT69L and saline self-infusion.

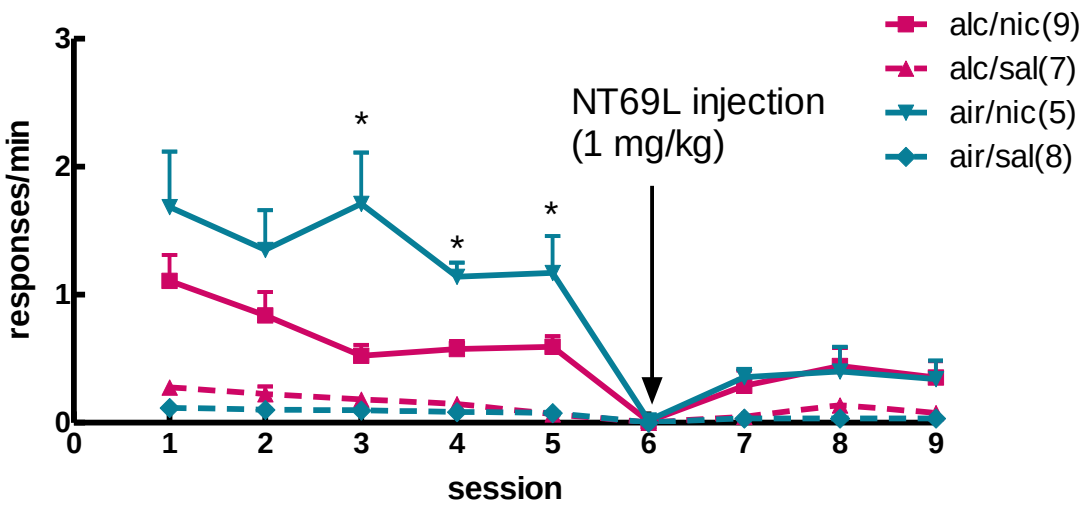


Figure 2
Chronological illustration of NT69L effect on nicotine self-infusion in alcohol- and air-exposed Wistar rats. The time course for nicotine self-infusion in alcohol- and air-exposed rats is indicated by each session. Pretreatment with a single dose of NT69L significantly attenuated nicotine self-infusion for 5 days post NT69L injection. Values represent the number of active nicotine self-infusions (mean ± SEM); * significantly different from corresponding saline groups ($p < .001$).

Withdrawal Studies

Effect of NT69L on nicotine withdrawal signs in rats pre-exposed to alcohol vapor or air. After two weeks of twice daily nicotine injections, the discontinuation of nicotine injections resulted in the development of acute nicotine somatic withdrawal signs. The rats exhibited behaviors including body shakes, chews, cheek tremors, eye blinks, foot licks, genital licks, head shakes, teeth chattering, and writhes. All responses were summed in a 10 minute observation period for each rat. The averages of all the rats' responses are shown in Figure 3. Discontinuation of nicotine treatment significantly increased withdrawal signs, $F(5, 62) = 80.869$, $p < .001$. Both alcohol and air exposed groups showed significantly higher withdrawal responses as compared to pre-nicotine treatment (baseline) ($p < .001$). Alcohol vapor exposed rats showed significantly lower withdrawal signs than the air exposed group ($p < .001$).

Pretreatment with NT69L 30 minutes prior to observation attenuated the nicotine withdrawal signs ($p < .001$) to levels that were not significant from pretreatment values in the air exposed group ($p = .531$) and the alcohol exposed group ($p = .640$). Figure 4 shows the withdrawal signs expressed as percent (%) change from baseline.

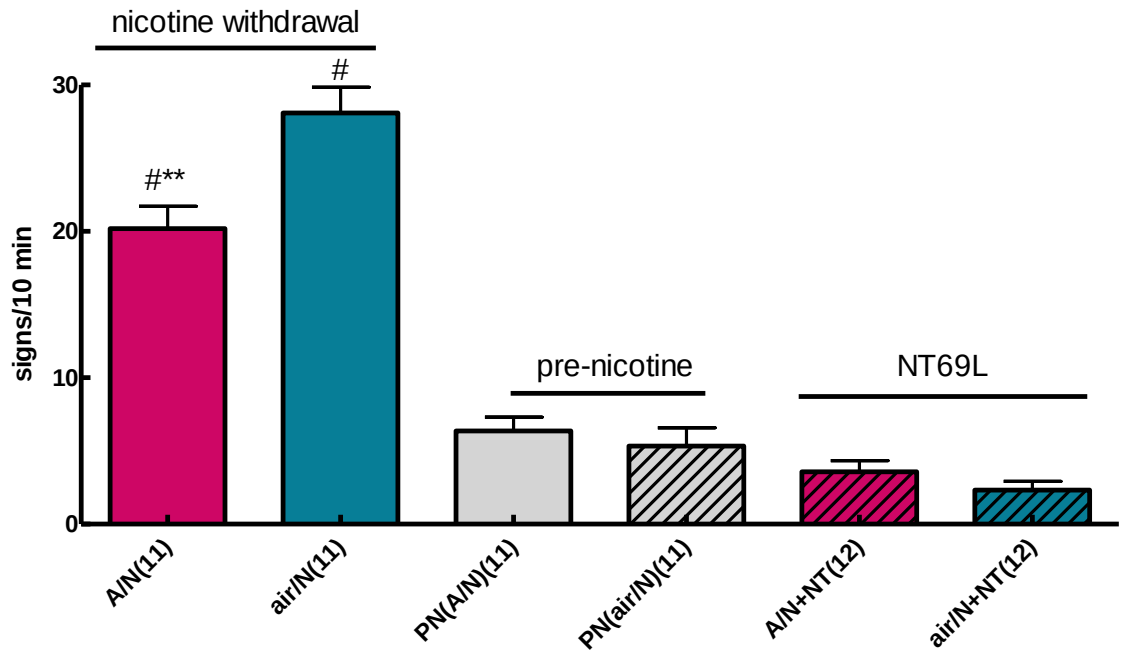


Figure 3
Effect of NT69L on nicotine withdrawal signs in alcohol- and air-exposed Wistar rats.
Alcohol- and air-exposed rats were injected twice daily for 15 days with nicotine (0.35 mg/kg s.c.). On day 15 the nicotine injections were discontinued and the animals were injected with either NT69L (1mg/kg i.p.) or saline. Withdrawal signs were recorded 30 minutes post NT69L or saline injection and depicted as total signs /10 minutes. (n), number of animals in each group. ** Significantly different from air/N ($p < .001$); # significantly different from respective NT69L and pre-nicotine (PN, baseline) groups ($p < .001$).

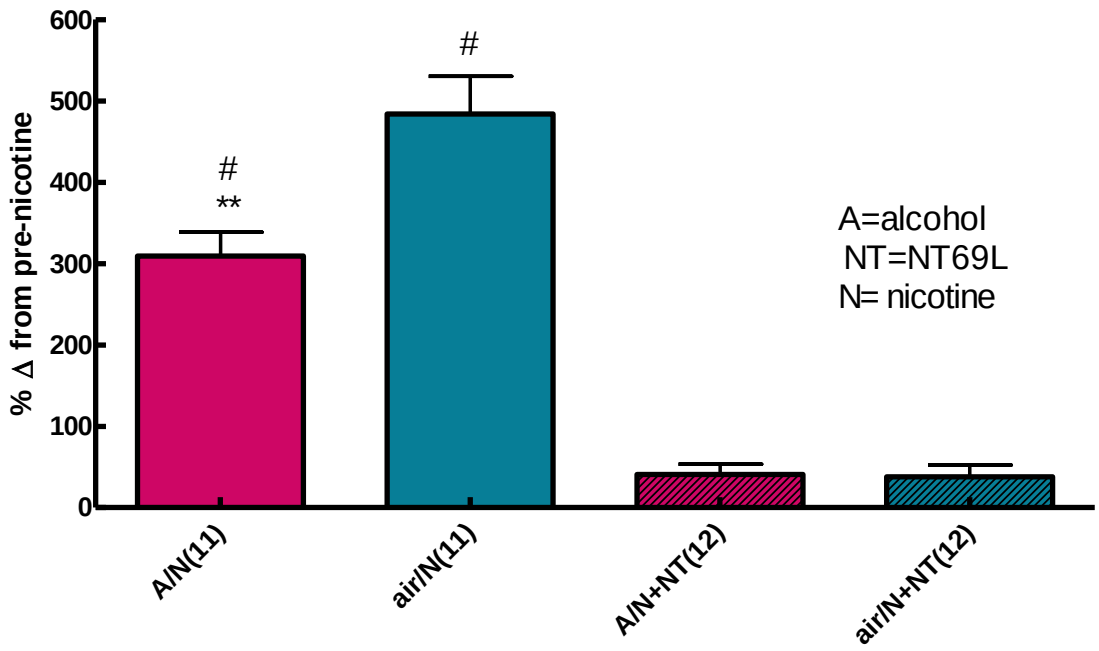


Figure 4
Percent (%) change from baseline measures of nicotine withdrawal signs in alcohol- and air-exposed Wistar rats.
Alcohol- and air-exposed rats were injected twice daily for 15 days with nicotine (0.35 mg/kg s.c.). On day 15 the nicotine injections were discontinued and the animals were injected with either NT69L (1mg/kg i.p.) or saline. Withdrawal signs were recorded 30 minutes post NT69L or saline injection and depicted as the percent of change from pre-nicotine (baseline) measures**.** ** Significantly different from air/N ($p < .001$); # significantly different from corresponding NT69L and pre-nicotine (PN/ baseline) groups ($p < .001$).

Effect of NT69L on alcohol withdrawal signs in rats pre-exposed to alcohol vapor or air. Acute alcohol withdrawal following 3 weeks of exposure to alcohol vapor resulted in the development of alcohol withdrawal signs. Displayed in Table 2, the rats that were exposed to alcohol vapor showed wet dog shakes and body tremor behavior as well as exhibiting straub tail upon acute alcohol withdrawal. Additionally, Figure 5 shows that the acute alcohol withdrawal resulted in increased locomotor activity, $F(4, 34) = 38.305$, $p < .001$. In Figure 6, hyperalgesia (excessive sensitivity to pain), as measured by reduced tail flick latency to radiant heat in an Analgesia Meter, was induced by alcohol withdrawal in alcohol exposed rats as compared to air

exposed rats, $F(4, 35) = 7.738$, $p < .001$. Increased catatonia (muscle rigidity) was seen in the alcohol exposed rats, $F(3, 22) = 47.899$, $p < .001$, as shown in Figure 7. As depicted in Figure 8, alcohol exposed rats showed a lowered body temperature (hyperthermia) from baseline, $F(3, 22) = 12.850$, $p < .001$.

Table 2: Effect of NT69L on observed acute alcohol withdrawal signs

Withdrawal sign (10 min observation period)	Alcohol	Air	Alcohol+NT69L	Air+NT69L
Wet dog shake	6.33±0.76**	0.0	0.0	0.0
Tremors	1.17±0.47*	0.0	0.0	0.0
Straub tail	+ + + +	–	–	–

Significantly different from air exposed group and NT69L pretreatment (**$p < .001$; *$p = .035$)

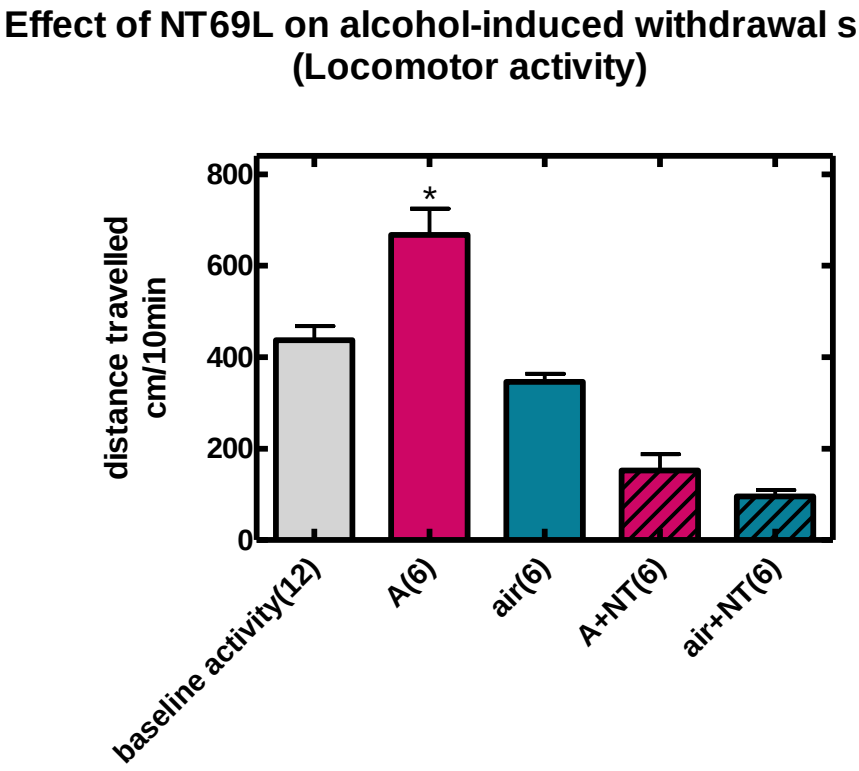


Figure 5
Effect of NT69L on alcohol withdrawal-induced hyperactivity
Activity was recorded in a Plexiglas Opto-Varimex activity chamber equipped with infrared photocell emitters and detectors. Baseline activity was recorded. Male Wistar rats were then exposed to alcohol (14h on/10h off) or air for 3 weeks. Alcohol exposure was discontinued and 2 hours after anticipated start of alcohol vapor exposure the rats were injected with either NT69L (1mg/kg i.p.) or saline (i.p.). Activity was recorded every 10 minutes for a 2 hour period. Data is reported as the distance traveled in cm per 10 minutes. * Significantly different from all other treatment ($p < .05$). A = alcohol, NT = NT69L

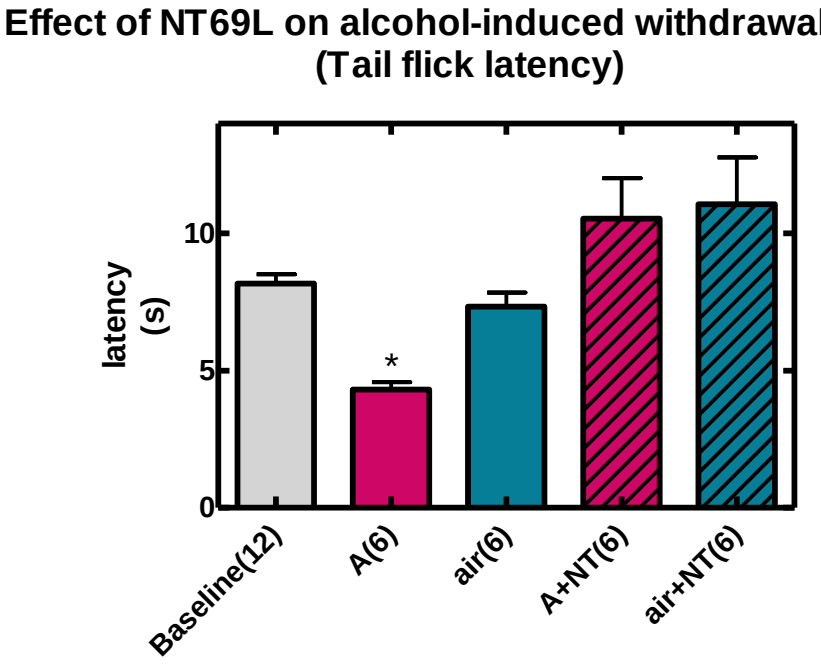


Figure 6
Effect of NT69L on alcohol withdrawal-induced hyperalgesia
Male Wistar rats were exposed to alcohol (14h on/10h off) or air for 3 weeks. Alcohol exposure was discontinued and 2 hpurs after anticipated start of alcohol vapor exposure the rats were injected with either NT69L (1mg/kg i.p.) or saline (i.p.). Hyperalgesia (excessive sensitivity to pain) was recorded with the use of an Analgesia Meter which assed the tail flick latency to radiant heat. Data is reported as the latency in seconds for the rat to flick its tail. Baseline measures were taken prior to alcohol exposure. * Significantly different from all other treatment ($p < .05$). A = alcohol, NT = NT69L

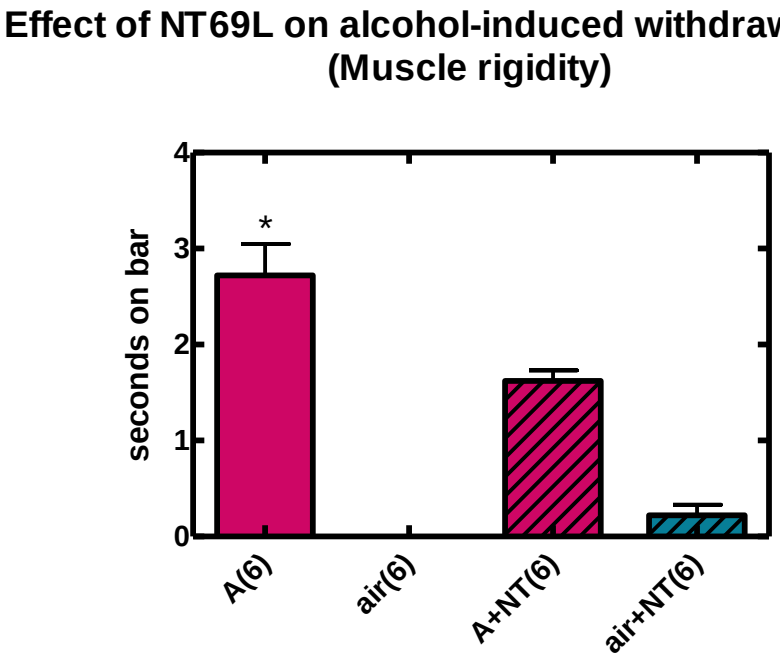


Figure 7
Effect of NT69L on alcohol withdrawal-induced catatonia (muscle rigidity)
Male Wistar rats were exposed to alcohol (14h on/10h off) or air for 3 weeks. Alcohol exposure was discontinued and 2 hours after anticipated start of alcohol vapor exposure the rats were injected with either NT69L (1mg/kg i.p.) or saline (i.p.). Increased catatonia was measured by the bar test 30 minutes post NT69L injection. The rats' front paws were placed on a suspended metal bar. Data is presented in the latency in seconds for the rat to remove its paws from the bar. Three measures were taken per rat and the average time in seconds is reported. * Significantly different from all other treatment ($p < .05$). A = alcohol, NT = NT69L

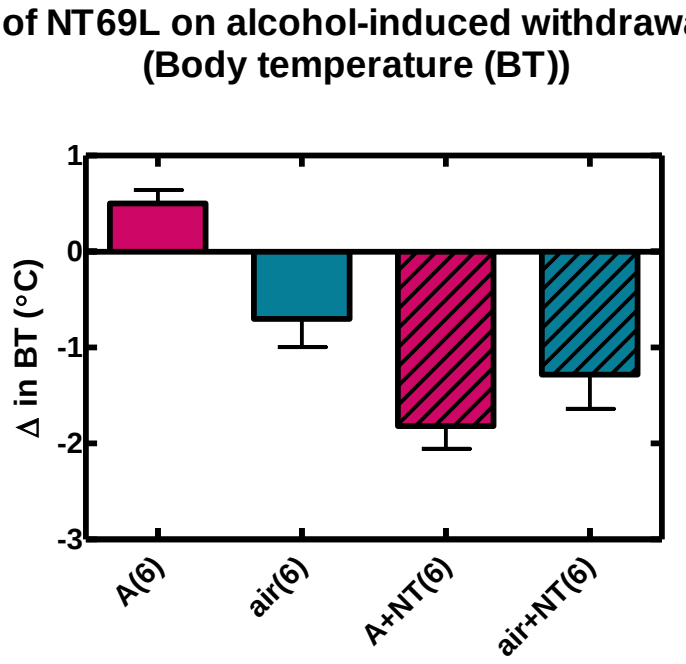


Figure 8
Effect of NT69L on alcohol withdrawal-induced change in body temperature (hypothermia)
A baseline body temperature reading (ºC) was recorded prior to alcohol exposure. Male Wistar rats were exposed to alcohol (14h on/10h off) or air for 3 weeks. Alcohol exposure was discontinued and 2 hours after anticipated start of alcohol vapor exposure rats were injected with either NT69L (1mg/kg i.p.) or saline (i.p.). 30 minutes later, animals were measured for body temperature by means of a thermistor probe inserted approximately 2 cm into the rectum of the rat. Data is presented as the change in body temperature from the baseline temperature reading in ºC
A = alcohol, NT = NT69L

Blood alcohol levels (BALs)

The figure below (Figure 9) shows the blood alcohol levels in the alcohol exposed and air groups. The alcohol group was exposed to alcohol vapor for 3 weeks (14h on/ 10h off) as described previously. The control group was exposed to air. The alcohol-exposed group has significantly higher levels of alcohol as compared to the air exposed group ($p < .001$).

The target range for blood alcohol levels (BALs) during vapor exposure was from 100 to 150 mg/100 ml (Roberts, Cole, & Koob, 1996; O'Dell et al., 2004). Blood alcohol levels are considered undetectable (< 20mg/100 ml) in nondependent (control) animals (Gilpin et al.,

2009). Blood alcohol levels were 10.26±0.83 and 126.4±1.85 mg/dL for the air and alcohol exposed rats respectively.

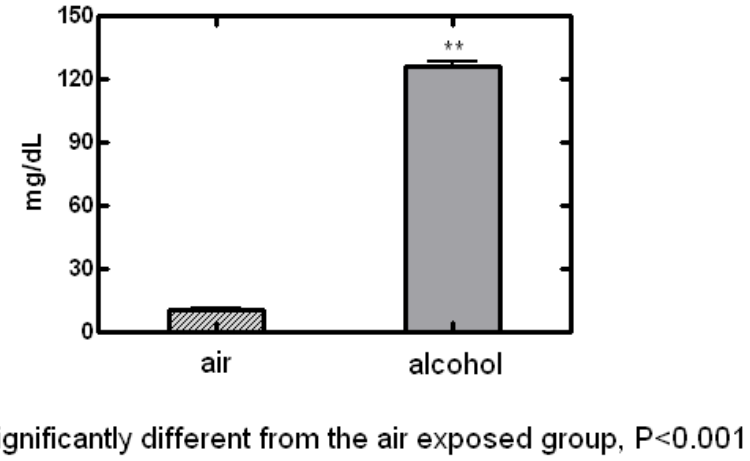


Figure 9
Blood alcohol levels (BALs) in air or alcohol-exposed Wistar rats
Blood alcohol levels were measured with the use of an Analox AM1 analyzer and reported as mg/dL.
**Significantly different from the air exposed group ($p < .001$)

Discussion

Nicotine and alcohol interact in their rewarding effects (Lê et al., 2010). The area of the brain responsible for the reinforcing properties of drugs such as alcohol, nicotine, cocaine and opiates, is the nucleus accumbens (Pontieri et al., 1996). Nicotine and alcohol stimulate a release of dopamine in the nucleus accumbens and both have a very strong dependence liability (Levinthal, 2010; Rang et al., 2003).

One of the leading causes of preventable death in the world is smoking (Jupp & Lawrence, 2010). Approximately 8.5% of adults in the U. S. (about one in twelve), are either alcohol abusers or alcohol dependent according to the *DSM-IV-TR* (2000) standards. Epidemiological and clinical studies estimated that 50 to 80% of alcohol-dependent individuals are regular smokers (Hurt et al., 1996; Romberger & Grant, 2004). Consequently, humans that co-abuse alcohol and nicotine have a worse clinical outcome than individuals who use only one of the substances (Lajtha & Serchen, 2010).

Cigarette smoking is highly prevalent among people with alcohol use disorders such as alcohol abuse and dependence. Smoking rates in treatment-seeking populations may be as high as 80% (Hughes, 1996). Given the increased nicotine-related mortality and morbidity in alcohol-dependent smokers (Hurt et al., 1996), and the greater difficulty in quitting smoking, it is critical to identify the most effective treatment possible. Currently, there are drugs available that target specifically nicotine dependence or alcohol dependence alone. For the treatment of nicotine addiction, several pharmacotherapeutic options have been approved by the USFDA. Nicotine replacement therapy is the main pharmacological approach used for treatment (Jupp & Lawrence, 2010). Despite various evidence found on the efficacy of nicotine replacement therapy, attempts at long-term cessation succeed in only about 20% of the cases (Rang et al.,

2003). Similar to nicotine addiction, alcoholism is very difficult to treat. Despite the availability of multiple different therapeutic options for the treatment of alcoholism and the utility they offer, the treatment effect size remains small (Jupp & Lawrence, 2010). There are no therapeutic drugs available on the market for the co-dependence of nicotine and alcohol. Consequently, there is a critical need for the development of novel drugs implementing new therapeutic targets that will help with smoking cessation and alcohol dependence.

NT69L is a neurotensin receptor agonist that attenuates nicotine self-administration in rats (Boules et al., 2011) and alcohol consumption in mice (Lee et al., 2010). NT69L works by interacting with the common neurotransmitter circuits supporting the rewarding process for both nicotine and alcohol which proves that it is a good candidate for the treatment of nicotine dependence in alcohol dependent rats.

Effect of NT69L on nicotine self administration in alcohol dependent rats

The most important finding in this study is that NT69L significantly attenuated nicotine self-administration in alcohol dependent rats as well as in air exposed rats. The alcohol dependent animals had an average BAL of 126.4 mg/dL. The target range for blood alcohol levels (BALs) in rats to induce alcohol dependence by vapor exposure is from 100 to 150 mg/dL (O'Dell et al., 2004; Roberts et al., 1996). Blood alcohol levels are considered undetectable (< 20mg/dL) in nondependent (control) animals (Gilpin et al., 2009). These blood alcohol levels can be related to human behavior at different levels of intoxication. In a study of U.S. drivers, the probability of being involved in an accident is unaffected at BALs up to 50 mg/dL. The chance of being involved in an accident increases by fourfold with a blood alcohol level of 80 mg/dL. By BALs at 150 mg/dL, the probability is increased by 25-fold. In another study, 'gross intoxication' was evaluated by a battery of tests that measured things such as speech and gait. It

was reported that ‘gross intoxication’ occurred in 30% of subjects between 50 and 100 mg/dL and in 90% of subjects with more than 150mg/dL. Beyond those levels, coma generally occurs at about 300mg/dL and death from respiratory failure is likely at 400-500 mg/dL (Rang et al., 2003).

The results with the air exposed group of rats are similar to previous results from Boules et al. (2011). In addition, our results showed that the alcohol exposed rats self-administered less nicotine as compared with air exposed rats. These results are in agreement with a study done by Lê et al. (2010) where access to alcohol reduced nicotine self-administration in rats that readily administered alcohol orally and nicotine intravenously at the same time. Interestingly in contrast to these findings, a previous study using alcohol preferring (P) rats, Lê et al. (2006) found that alcohol preferring rats readily self-administered more nicotine as well as expressed greater nicotine-seeking behavior than the alcohol non-preferring (NP) rats. However, the difference between our data and those of Lê et al. (2006) is probably due to the genetic liability of the alcohol preferring rats, which are selected for high alcohol drinking due to the fact that genetic studies in humans have established that the liability to use either alcohol or nicotine alone or both drugs is co-inherited (Dani & Harris, 2005).

Effect of NT69L on nicotine and alcohol withdrawal

Nicotine withdrawal. Smoking cessation is known to produce an aversive withdrawal syndrome in humans (Hughes, 1996). The nicotine withdrawal syndrome includes increase nervousness, frustration, anger, and desire to smoke (Snyder et al., 1989). Nicotine withdrawal is also associated with somatic symptoms such as gastrointestinal discomfort, increased appetite, and other affective symptoms (depressed mood, anxiety, difficulty concentrating etc.) (4th ed., text rev.; *DSM–IV–TR*; American Psychiatric Association, 2000; Hughes, 1996). The aversive

aspects of nicotine withdrawal contribute to high relapse rates to tobacco smoking after cessation attempts (Skjei & Markou, 2003). The nicotine withdrawal syndrome is known to occur in both humans and experimental animals accustomed to regular nicotine administration (Rang et al., 2003). A discontinuation of chronic nicotine administration in rats results in the appearance of several somatic signs such as eye-blinks, body-shakes, gasps, foot licks, chews and abdominal writhes (i.e. "somatic signs of nicotine withdrawal") (Hughes, 1996; Skjei & Markou, 2003).

In addition to blocking the rewarding effects of nicotine, NT69L reversed withdrawal signs (induced by the cessation of nicotine administration) to levels that were similar to pre-nicotine treatment or baseline measures. Prior to treatment with NT69L, the alcohol- and nicotine-exposed group showed significantly lower levels of withdrawal signs than the nicotine only exposed group. The somatic nicotine withdrawal signs include body shakes, chews, cheek tremors, eye blinks, foot licks, genital licks, head shakes, teeth chattering, and writhes. This is in agreement with previous studies that show that NT69L effectively blocks the initiation and expression of nicotine-induced sensitization (Fredrickson et al., 2003a; Fredrickson et al., 2003b). Nicotine-induced sensitization is a mechanism that is thought to underlie craving and risk for relapse to smoking (Fredrickson et al., 2005).

Alcohol withdrawal. Alcohol withdrawal symptoms result from the discontinuation of alcohol use after prolonged exposure. Like with nicotine withdrawal, symptoms of alcohol withdrawal make abstaining from alcohol use very difficult. Withdrawal symptoms are a very influential factor leading to relapse of alcohol use in recovering alcoholics. Alcohol withdrawal in humans is classified into two clusters: alcohol withdrawal syndrome and delirium tremens. The most commonly reported symptoms are tremors ("the shakes"), sweating, mild agitation, anxiety (the "jitters"), nausea, and vomiting. A similar syndrome can be produced in

experimental animals by ethanol withdrawal (Rang et al., 2003). The physical signs of alcohol withdrawal in rats include convulsions, motor abnormalities, and autonomic disturbances (Gilpin et al., 2009). In order of ascending severity, somatic withdrawal symptoms in rats include general hyperactivity, tail spasticity/tremors, general spasticity/tremors, head tremors, wet shakes, teeth chattering, and spontaneous tonic-clonic convulsions (Majchrowicz, 1975).

Matching the results on nicotine withdrawal signs, NT69L significantly reversed the physical signs of alcohol withdrawal in rats. NT69L significantly decreased the presence of somatic signs including wet dog shakes, tremors, and straub tail in the alcohol-induced withdrawal animals. Also, NT69L exhibited its effect on alcohol induced hyperactivity, hyperalgesia, catatonia, and body temperature. Alcohol withdrawal induced hyperactivity in rats. After pre-treatment with NT69L, activity levels were significantly lowered. The excessive sensitivity to pain, hyperalgesia, was significantly lowered by NT69L proving its' analgesic effect and muscle rigidity (catatonia) was reduced in alcohol-withdrawing animals after treatment with NT69L as well.

In summary, pretreatment with a single injection of the neurotensin agonist, NT69L, significantly attenuated nicotine self-administration in rats in both alcohol-dependent and air exposed groups. Alcohol dependence did not increase nicotine self-administration in rats. The lack of effect from alcohol dependence on nicotine self-administration might be due to the fact that the rats were passively exposed (vapor) to alcohol which removed the conditioning effect of drinking and smoking in humans. This conditioning might be the reason why alcoholics tend to smoke more than non-alcoholic individuals. Nonetheless, NT69L reduced nicotine self-administration in both the alcohol and air exposed groups as expected. Also as expected, pretreatment with NT69L significantly decreased nicotine withdrawal signs in alcohol dependent

and non-alcohol dependent rats. Withdrawal signs from alcohol were also significantly reduced in alcohol-dependent rats pretreated with NT69L. Neurotensin agonists, particularly NT69L, may represent a potential novel pharmacotherapeutic option for the treatment of combination alcohol and nicotine addiction in humans.

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Publications

Blashfield, R. K., Reynolds, S. M., Stennett, B. A. (2012). The death of histrionic personality disorder. In T.A. Widiger (Ed.), *Oxford Handbook of Personality Disorders.* New York: Oxford University Press.

Posters

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