

**Combination Therapies of the Neurotransmitter-Targeting Drugs
Dextromethorphan, Pyrilamine and Lorcaserin in a Rat Model of
Nicotine Addiction**

Scott Alan Briggs

A thesis submitted to the Department of Biology for distinction

**Duke University
Durham, North Carolina
2014**

Introduction

Since 1964, cigarette smoking and tobacco smoke pollution have caused the premature deaths of an estimated 14 million Americans in addition to substantially more around the world, making the dependence on nicotine consumption a very important matter of public health (Giovino 2007). It is estimated that tobacco use is responsible for almost 20 percent of all premature deaths in developed countries, making it the single largest cause of premature death worldwide (Dani & Heinemann 1996). Lung cancer is the leading cause of death among smokers, but other major risks associated with smoking include other forms of cancer, heart disease, other cardiovascular diseases, chronic respiratory diseases, pregnancy complications, and respiratory diseases in children due to second-hand smoking. Although per capita American tobacco consumption has declined significantly since the 1950s, as of 2005 one in five Americans still classified themselves as smokers (Giovino 2007). Despite a wide variety of current treatments for smoking cessation including nicotine patches, nicotine gum, nicotine sprays, and smokeless cigarettes, nicotine addiction remains one the most difficult drug addictions to treat. Only three to five percent of cigarette smokers who attempt to quit are able to remain abstinent for six to twelve months (Hughes et al 2004). Most smokers fails in their attempts to quit smoking within eight days, and it takes the average smoker numerous attempts to successfully quit smoking, if he or she ever succeeds in quitting at all (Hughes et al 2004).

Neurological Basis for Nicotine Addiction

The mechanisms underlying the development of addiction are complex and include many neurotransmitter interactions involved in pleasure, motivation, and learning. Principal among these is the dopamine circuit with projections from the ventral tegmental area in the midbrain to the nucleus accumbens in the forebrain, often called the reward pathway (Cami & Farre 2003).

Although the process is not completely understood, it is believed that nicotine addiction is mediated by the binding of nicotine molecules with nicotinic acetylcholine receptors on dopamine neurons in the ventral tegmental area. The binding of nicotine with these receptors, which are the main binding sites for nicotine in the body and are responsible for the regulation of a wide variety of bodily functions, leads to the downstream release of excess dopamine levels in the nucleus accumbens (Rice & Cragg 2004). In addition to this release of dopamine that is primarily responsible for the development of nicotine addiction, nicotinic acetylcholine receptor activation exhibits its many other effects on the body through excitatory postsynaptic responses at glutamate, GABA, and serotonin terminals (Dani 2001). The release of these other neurotransmitters occurs through a complex network of interacting neurotransmitter pathways, but the specific details of these pathways are not well understood (Levin et. al. 2008). Elucidating a better understanding of these neurotransmitter interactions has the potential to result in novel approaches for treating nicotine addiction.

Histamine Antagonism and Pylramine

In 1995, a serendipitous observation led to the discovery that the antipsychotic clozapine significantly reduced smoking in patients with schizophrenia (McEvoy et. al. 1995). Subsequent studies have found that, in normally functioning rats, moderate doses of clozapine exerts these effects by impairing working memory and blocking nicotine-induced memory and attentional improvement (Levin et. al. 2007). Clozapine is a complex drug that acts as an antagonist at a variety of neurotransmitter receptors such as those for dopamine, serotonin, norepinephrine, and histamine.

Following these discoveries, multiple studies have been conducted in an effort to better elucidate by which of these neurological pathways clozapine exerts its smoking-reducing effects.

Pyrilamine, an H1 histamine antagonist that is often used as an antihistaminic agent in over-the-counter cold medication, recently has been shown to decrease nicotine self-administration in rats (Levin et. al. 2011). Although pyrilamine significantly reduces nicotine self-administration independently, it does not completely counteract the neural actions that underlie continued nicotine consumption, nor does it halt nicotine self-administration entirely. A combination therapy including drugs targeting other neurotransmitters may result in more beneficial interactions in the reward pathway, which could lead to greater and more significant reductions in nicotine self-administration. A discovery of such interactions in combination therapy may lead to novel treatments for nicotine addiction and smoking cessation.

Serotonin Agonism and Lorcaserin

Lorcaserin, a selective 5-HT_{2C} receptor agonist currently approved in the United States as a weight loss drug, also recently has been shown to decrease nicotine self-administration in rats (Higgins et. al. 2012) and attenuate other drug addictions in humans (Cunningham et. al. 2011). Bubar and Cunningham identify the role of 5-HT_{2C} receptor activation in decreasing dopamine release as the principal mechanism by which lorcaserin and similar serotonin agonists reduce the reinforcing behavior critical in drug addiction (2006). Since serotonin plays a significant role in the reward pathway and lorcaserin has previously been shown to decrease nicotine administration independently, there is reason to believe that a combination therapy including drugs targeting other neurotransmitters may result in greater attenuation of nicotine self-administration.

Glutamate Antagonism and Dextromethorphan

Dextromethorphan, an over-the-counter antitussive agent that can act as a dissociative hallucinogen at high doses, has recently been shown to reduce nicotine self-administration in rats

(Glick et. al. 2001). Dextromethorphan and its principle metabolite dextrorphan have many mechanisms of action. It may act as a nonselective serotonin reuptake inhibitor, sigma-1 receptor antagonist, and NMDA glutamate receptor antagonist. It has also been shown that dextromethorphan can act as a noncompetitive alpha-3 beta-4 nicotinic antagonist. (Hernandez et al 2000). This mechanism of action, in combination with the NMDA glutamate antagonism that suppresses reinforcing drug effects, is believed to be responsible for dextromethorphan's ability to reduce nicotine self-administration.

Potential Interactions of Dextromethorphan, Pyrilamine, and Lorcaserin

In this study, we test two drug combinations, 1) dextromethorphan and pyrilamine and 2) dextromethorphan and lorcaserin, in a rat model of nicotine addiction to determine if any significant neurotransmitter interactions between the two drugs exist. I believe that by combining two drugs targeting neurotransmitter receptors involved in the reward pathway, additive or synergistic effects may result in more significant levels of nicotine self-administration reduction compared to the administration of either drug independently, and potentially elucidate more details about the reward pathway.

In this study, we tested combinations of placebo, low, and high doses of 1) dextromethorphan and pyrilamine and 2) dextromethorphan and lorcaserin in rats to determine interaction effects in the following:

- 1) Nicotine self-administration as a model of nicotine addiction.
- 2) Locomotor activity as a measure of drug sedating and stimulating effects.
- 3) Food pellet self-administration as a measure of drug effects on appetite and as a secondary measure of drug sedating and stimulating effects.

While effects on nicotine self-administration are the primary concern for this study, effects on locomotor activity and food pellet self-administration are important control measures to ensure that reduction in nicotine self-administration is not a result of general sedation caused by one or both drugs.

Methods

Animal Subjects

Young adult female rats were used for this study. Each rat used for this study was housed in a separate, properly bedded chamber in standard laboratory conditions approved by Duke University. Care was taken to transport the subjects through the laboratory with minimal stress. To ensure that rats were awake and in an active phase during experimental sessions, the housing room was kept in a reverse day/night cycle. Rats were consistently fed following experimental sessions and were given constant access to water through standard cage bottles or through automatic delivery lines in housing room racks. The rats were housed and cared for in conditions in accordance with all university, state, and federal regulation.

Locomotor Activity

Locomotor activity of the subjects was observed using a Figure-8 maze, which was first demonstrated as a measure of locomotor activity by Norton et. al. (1975). As described in Ruppert et. al. (1985), this maze is a continuous enclosed alley (10 cm by 10 cm) in the shape of a Figure-8 (70 cm long and 42 cm wide with a 21 cm by 16 cm central arena) with two blind alleys extending 20 cm from either side. Eight infrared photobeams crossing the maze alleys are used to measure locomotor activity, one located on each of the two blind alleys and three on each of two loops of the Figure-8. Activity was determined based on the number of breaks in the photobeams over one hour, and trends in locomotor activity were analyzed to test for potential locomotor effects of the drugs dextromethorphan, pyrilamine, and lorcaserin (Timofeeva et al 2008). Following four days of acclimation, rats were tested once for baseline locomotor activity without any drug injections prior to entering the maze. Over the next nine days, rats were tested with each combination of dextromethorphan (0, 10, 30 mg/kg) and pyrilamine (0, 13.3, 40

mg/kg) or dextromethorphan (0, 10, 30 mg/kg) and lorcaserin (0, 0.625, 1.25 mg/kg), injected 10 minutes prior in randomized chronological order.

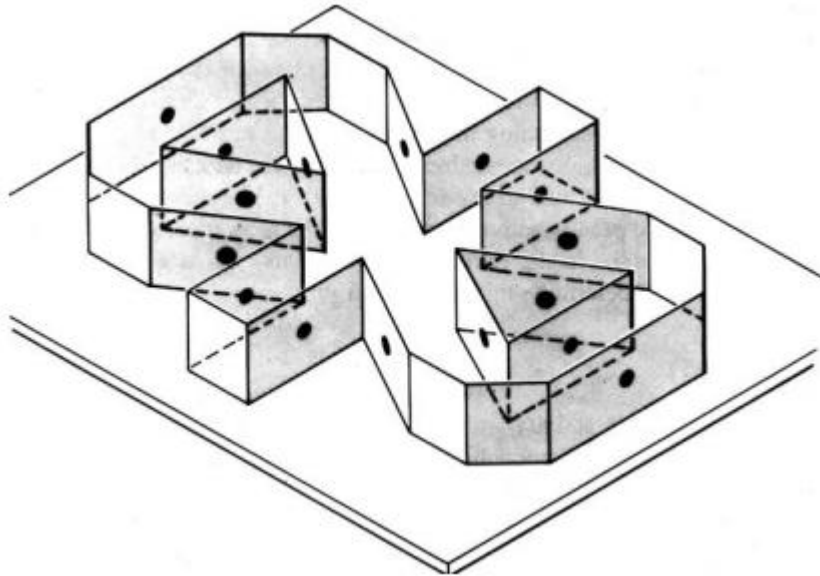


Figure 1: Figure-8 maze. The location of photocells (n=8) that detect motor activity is indicated by dark circles. (Reprinted from Ruppert et al 1985.)

Subject Training

Prior to jugular catheterization surgery and nicotine self-administration sessions, rats must be trained to use the lever-press self-administration chambers purchased from MED Associates, Inc. Rats are placed in separate, dual operant chambers, having been in the absence of food for at least twelve hours. These chambers have one active and one inactive lever. The active lever is vertically paired with a cue light which illuminates when a food pellet is available. When the lever is pressed, a food pellet is delivered via a feeding tray, a .5s feedback tone is sounded, and the light goes out until another food pellet is available via lever press. The inactive lever does not have an illuminated cue light, and does not have any effect if pressed. Prior to training sessions, rats are placed in chambers for two overnight sessions, during which the rats are periodically delivered food pellets paired with illumination of the cue light until the rat learns

to associate the illuminated lever with food pellet delivery. A rat passes an overnight session when it successfully presses the active lever 100 times. Following overnight sessions, the rat must self-administer at least 50 food pellets during three 30-minute pellet sessions (P1-P3) in the absence of the overnight, automatic pellet delivery to be considered successfully trained.

Food Self-Administration

After the completion of locomotor activity sessions and pellet session training, rats continued with 30-minute food pellet self-administration chamber sessions in the presence of dextromethorphan and pyrilamine or dextromethorphan and lorcaserin to determine potential effect and interactions of these drugs on appetite and food self-administration at various doses. Over nine days, rats were given each combination of dextromethorphan (0, 10, 30 mg/kg) and pyrilamine (0, 13.3, 40 mg/kg) or dextromethorphan (0, 10, 30 mg/kg) and lorcaserin (0, 0.625, 1.25 mg/kg) injected 10 minutes prior to pellet sessions in randomized chronological order.

Preparation of Nicotine Solution

During self-administration, nicotine is administered to rats from lines attached through delivery ports into silicone-rubber catheter tubing implanted into the jugular vein. Solutions of 0.03mg/kg nicotine bitartrate are prepared with appropriate masses of nicotine salt. Using contaminate-free glassware, the nicotine salt is dissolved in sterile saline and adjusted to a standard pH between 7.0 and 7.20 using varying molar concentrations of HCl and NaOH in saline. After being adjusted to a pH appropriate for intravenous infusion, the nicotine solution is filtered through a Nalgene filter to ensure proper sterilization. These solutions are stored in conical tubes covered in aluminum foil to ensure limited exposure to light. Between sessions nicotine solutions are refrigerated for no longer than two weeks before replacement.

Jugular Catheterization Surgery

Following successful completion of overnight and pellet training sessions, jugular catheterization surgery is performed in a sterile, aseptic environment. In preparation for surgery, a general anesthesia mix of the drugs medetomidine and ketamine is delivered via injection. The rat is then shaved and prepared for surgery. Following incision and separation of tissue, the jugular vein is tied off at the distal end of the cannula insertion area. The catheter is inserted into the jugular vein just anterior to the heart and secured using cyanoacrylate adhesive. The external section of the catheter is sutured to the deep muscle and exits the body on the dorsal side of the rat just posterior to the scapulae. The catheter is held in place by a plastic delivery port and rubber underarm bands. The catheter is flushed with the anticoagulant heparin in sterile saline and the antibiotic gentamicin to prevent coagulation and infection following surgery.

Nicotine Self-Administration Procedure

Following jugular catheterization, rats transition from self-administration of food pellets to nicotine by a similar mechanism. The same delivery chamber is used, and the lever that previously administered a food pellet when pressed now delivers a 0.03 mg/kg/infusion dose of nicotine solution via the delivery line and catheter. As before, the opposing lever has no effect. Following each lever press and nicotine delivery, the cue light turns off for one minute, the house light turns on, and the lever is inactivated until the cue light illuminates again.

Experimental Design

The rats are first exposed to five baseline nicotine self-administration sessions in the absence of dextromethorphan, pyrilamine, or lorcaserin. Before nicotine self-administration sessions, catheters are flushed with 0.3 ml of a 100units/ml heparinized saline solution. These sessions last for 45 minutes, and responses are measured using MED-PC software. The software measures correct and incorrect lever presses, total nicotine infusion, and 15-minute interval

counts for nicotine infusion. Following sessions, nicotine is drawn out of the delivery port and replaced with 0.3 ml of a saline solution containing 8mg/ml of the antibiotic gentamicin and 500units/ml of heparin. After the completion of nicotine-only baseline sessions, each dose combination of dextromethorphan (0, 3.3, 10 mg/kg) and pyrilamine (0, 4.43, 13.3 mg/kg) or dextromethorphan (0, 3.3, 10 mg/kg) and lorcaserin (0, 0.3125, 0.625 mg/kg) is administered via sub dermal injection 10 minutes prior to sessions using a repeat counterbalance format discussed below. As explained in the Results, following the results of the locomotor activity and pellet sessions, the dose ranges of the drugs for the nicotine self-administration trials were reduced.

Repeated Measures Counterbalanced Design

The repeated measures counterbalanced design was used to reduce the possibility that the order of dosage administration or other factors could impact the results. In this design, each subject receives each of the nine dose combinations twice over a four-week period, with the order of dose combinations being administered varying across the subject pool. This way, every subject participates in every condition but the orders of doses are different and each subject can serve as its own control.

Data Analysis

Analysis of variance for repeated measures was conducted on the data and $p < 0.05$ was considered the threshold for statistical significance.

Results

Single Drug Effects on Locomotor Activity

Independently, all three drugs significantly decreased locomotor activity in a dose-dependent manner when administered ten minutes prior to locomotor activity session. The higher 30 mg/kg dose of dextromethorphan resulted in a significant decrease in locomotor activity relative to saline injection, while the lower 10 mg/kg dose did not result in a significant decrease (Fig. 2a). Both the higher 40 mg/kg dose and the lower 13.3 mg/kg dose of pyrilamine resulted in a significant decrease in locomotor activity relative to saline injection (Fig. 2b). Both the higher 1.25 mg/kg dose and the lower 0.625 mg/kg dose of pyrilamine resulted in a significant decrease in locomotor activity relative to saline injection (Fig. 2c).

Drug Interactions in Locomotor Activity

Significant interactions were observed with both drug combinations in locomotor activity trials. In the dextromethorphan-pyrilamine study, treatment with the lower 13.3 mg/kg dose of pyrilamine significantly interacted with both the lower 10 mg/kg dose and the higher 30 mg/kg dose of dextromethorphan in reducing locomotor activity by a greater margin than either dose alone (Fig. 3a). Treatment with the higher 40 mg/kg dose of pyrilamine also significantly interacted with the lower 10 mg/kg dose of dextromethorphan in decreasing locomotor activity. Treatment with the higher 40 mg/kg dose of pyrilamine appeared to interact with the higher 30 mg/kg dose of dextromethorphan to increase locomotor activity contrary to the observed trends; however, these results are not significant. In the dextromethorphan-lorcaserin study, treatment with the higher 1.25 mg/kg dose of lorcaserin significantly interacted with the higher 30 mg/kg dose of dextromethorphan in reducing locomotor activity by a greater margin than either dose

alone (Fig. 3b). Both doses of lorcaserin appeared to reduce locomotor activity with dextromethorphan in a dose-dependent manner, but these results were not significant.

Single Drug Effects on Food Self-Administration

Following the general trend observed with locomotor activity, all three drugs significantly decreased food pellet self-administration independently in a dose-dependent manner. The higher 30 mg/kg dose of dextromethorphan significantly decreased food self-administration relative to saline injection, while the lower 10 mg/kg dose did not (Fig. 4a). Both the higher 40 mg/kg dose and the lower 13.3 mg/kg dose of pyrilamine resulted in a significant decrease in food self-administration relative to saline injection (Fig. 4b). Both the higher 1.25 mg/kg dose and the lower 0.625 mg/kg dose of lorcaserin resulted in a significant decrease in food self-administration relative to saline injection (Fig. 4c).

Drug Interactions in Food Self-Administration

Significant interactions were only observed between dextromethorphan and pyrilamine in food pellet self-administration trials. Treatment with the lower 13.3 mg/kg dose of pyrilamine significantly interacted with both the lower 10 mg/kg and the higher 30 mg/kg doses of dextromethorphan in reducing food self-administration by a greater margin than either dose alone (Fig. 5a). Treatment with the higher 40 mg/kg dose of pyrilamine also significantly interacted with both the lower 10 mg/kg and the higher 30 mg/kg doses of dextromethorphan in reducing food self-administration. No significant interactions were observed between dextromethorphan and lorcaserin in food self-administration (Fig. 5b). Treatment with the lower 0.625 mg/kg dose of lorcaserin did appear to interact with both doses of dextromethorphan, but these results were not significant.

The low activity, or high sedation levels, observed with the high doses of dextromethorphan (30 mg/kg), pyrilamine (40 mg/kg), and lorcaserin (1.25 mg/kg) in the locomotor activity sessions and pellet sessions necessitated a decrease in the dose range used for nicotine self-administration. Following the locomotor activity and food pellet self-administration trials, the dose ranges were adjusted such that the old low dose became the new high dose for dextromethorphan (new range: 0, 3.3, 10 mg/kg), pyrilamine (new range: 0, 4.43, 13.3 mg/kg), and lorcaserin (new range: 0, 0.3125, 0.625 mg/kg).

Single Drug Effects on Nicotine Self-Administration

In the dextromethorphan-pyrimilamine study treatment with dextromethorphan ten minutes prior to nicotine self-administration sessions decreased nicotine self-administration in a dose-dependent manner; however, these results were not significant (Fig. 6a). In the dextromethorphan-lorcaserin study, however, treatment with dextromethorphan was shown to significantly decrease nicotine self-administration in a dose-dependent manner, as expected (Fig. 6b). In this study, both the lower 3.3 mg/kg dose and the higher 10 mg/kg dextromethorphan doses resulted in a significant decrease in nicotine self-administration relative to saline injection. As expected, treatment with both pyrilamine and lorcaserin ten minutes prior to nicotine self-administration sessions significantly decreased nicotine self-administration independently in a dose-dependent manner. The higher 13.3 mg/kg dose of pyrilamine significantly decreased nicotine self-administration relative to saline injection, while the lower 4.43 mg/kg dose did not (Fig. 6c). The higher 0.625 mg/kg dose of lorcaserin did result in a significant decrease in nicotine self-administration relative to saline injection, while the lower 0.3125 mg/kg dose did not (Fig. 6d).

Drug Interactions in Nicotine Self-Administration

Significant interactions in nicotine self-administration were only observed between dextromethorphan and lorcaserin. No significant interactions between dextromethorphan and pyrilamine were observed in the nicotine self-administration trials (Fig. 7a). Treatment with the higher 0.625 mg/kg dose of lorcaserin significantly interacted with the lower 3.3 mg/kg dose of dextromethorphan in reducing nicotine self-administration by a greater margin than either dose alone. Treatment with the lower 0.3125 mg/kg dose of lorcaserin also significantly interacted with the higher 10 mg/kg dose of dextromethorphan in reducing nicotine self-administration (Fig. 7b).

Figures

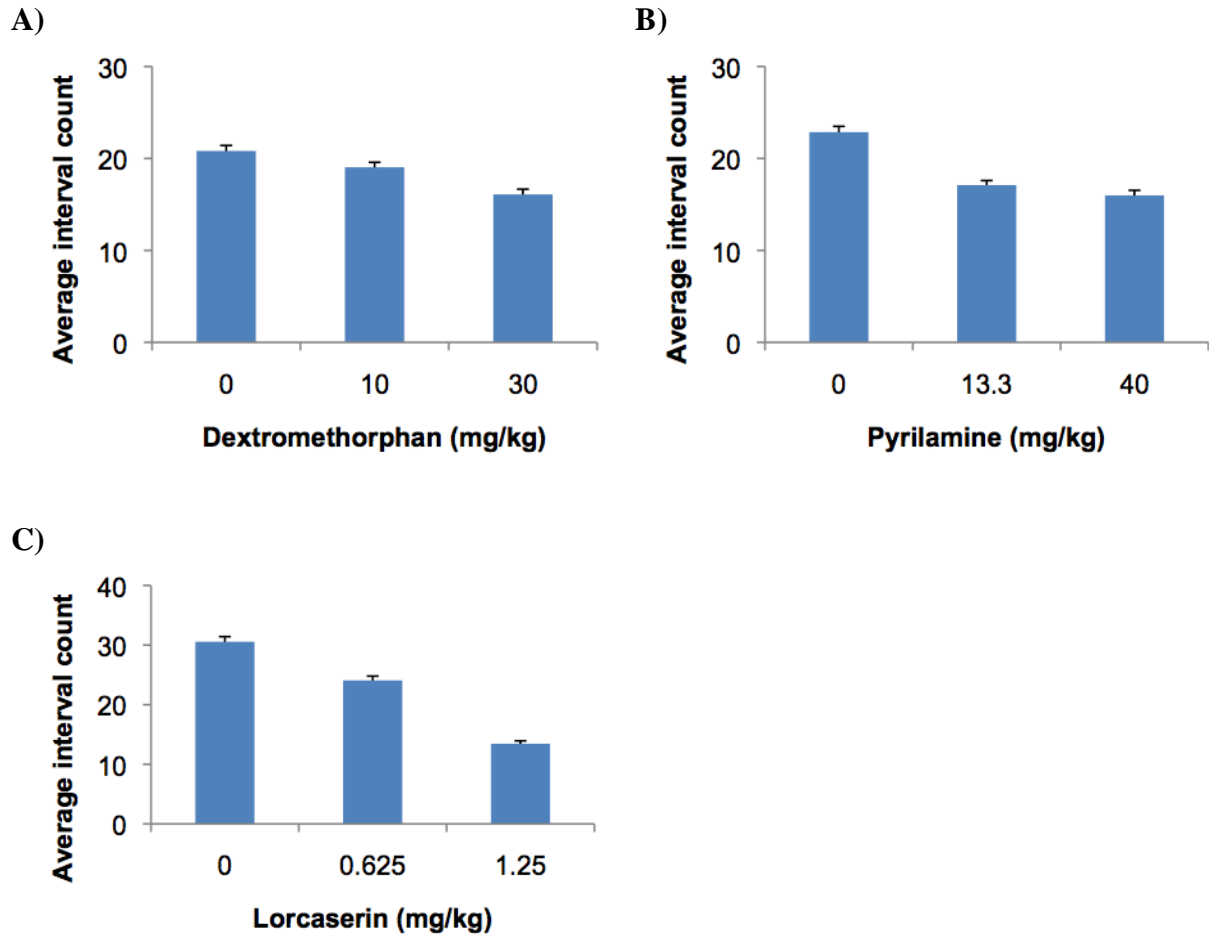


Figure 2: Single Drug Effects on Locomotor Activity

Average interval count refers to the average number of photobeam breaks per 5-minute time interval with each dose combination. **A)** In the dextromethorphan-pyrilamine study, treatment with dextromethorphan significantly decreased locomotor activity in a dose-dependent manner ($F(2,30)=10.268$, $p<0.0005$, $n=15$). The high dose of dextromethorphan did result in a significant decrease in locomotor activity relative to saline injection ($F(1,30)=20.118$, $p<0.005$, $n=15$). The low dose did not significantly decrease locomotor activity relative to saline injection ($F(1,30)=2.838$, $p>0.1$, $n=15$). Identical trends for effects of dextromethorphan alone on locomotor activity were observed in the dextromethorphan-lorcaserin study. **B)** Treatment with pyrilamine significantly decreased locomotor activity in a dose-dependent manner ($F(2,30)=15.247$, $p<0.0005$, $n=15$). Both the high and low doses of pyrilamine resulted in a significant decrease in locomotor activity relative to saline injection (High dose: $F(1,30)=26.481$, $p<0.005$, $n=15$) (Low dose: $F(1,30)=18.559$, $p<0.005$, $n=15$). **C)** Treatment with lorcaserin did significantly decrease locomotor activity in a dose-dependent manner ($F(2,30)=62.315$, $p<0.0001$, $n=15$). Both the high and low doses of lorcaserin resulted in a significant decrease in locomotor activity relative to saline injection (High dose: $F(1,30)=50.97$, $p<0.0005$, $n=15$) (Low dose: $F(1,30)=7.43$, $p<0.01$, $n=15$).

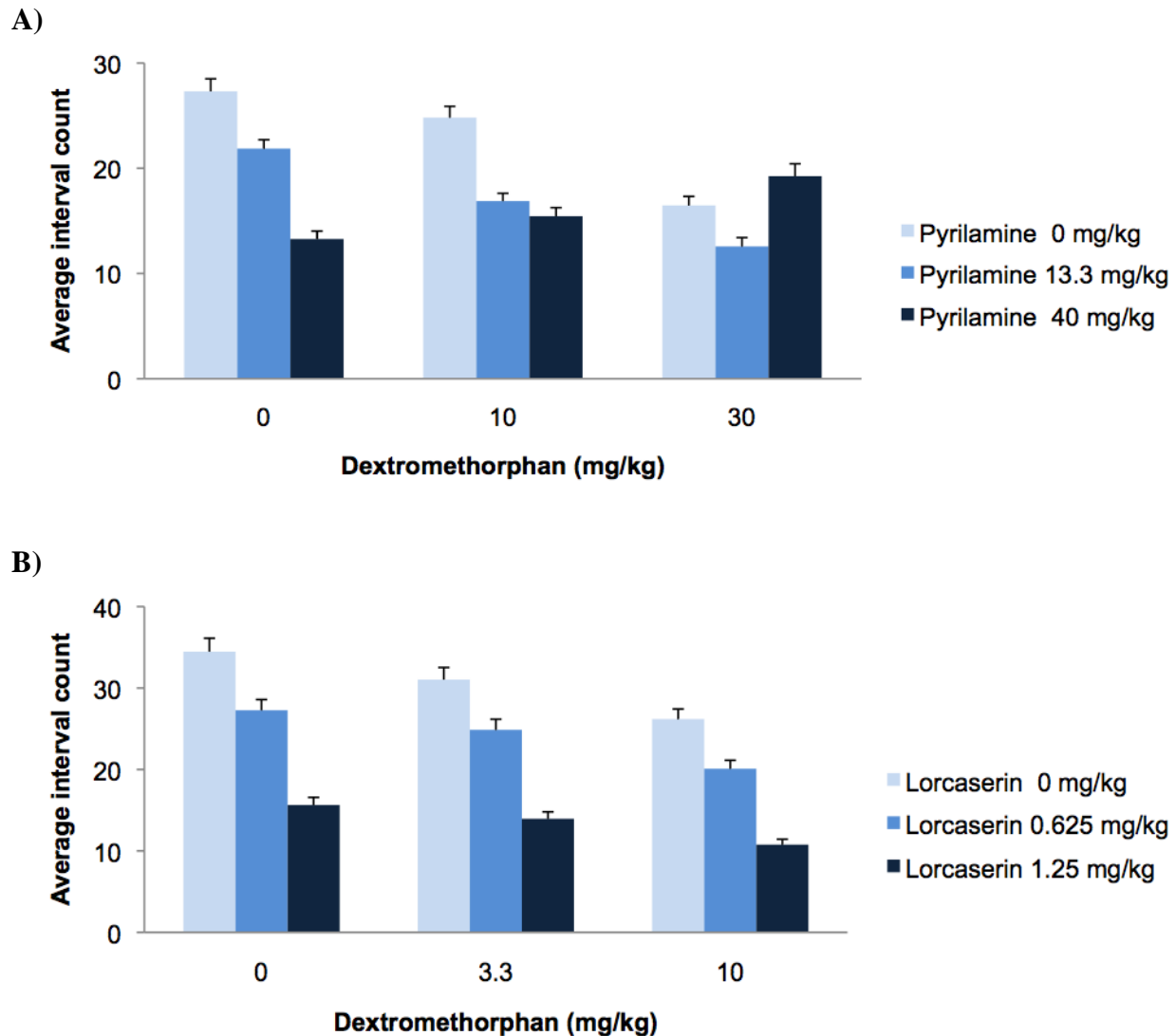


Figure 3: Drug Interactions in Locomotor Activity

Average interval count refers to the average number of photobeam breaks per 5-minute time interval with each dose combination. **A)** Treatment with the low dose of pyrilamine did significantly interact with both the low and high dose of dextromethorphan, resulting in greater reductions in locomotor activity than with either dose alone (Low dose: $F(1,30)=17.620$, $p<0.005$, $n=15$) (High dose: $F(1,30)=24.553$, $p<0.005$, $n=15$). Treatment with the high dose of pyrilamine resulted in significant interactions with the low dose of dextromethorphan ($F(1,30)=4.233$, $p<0.05$, $n=15$). Treatment with the high dose of pyrilamine appears to interact with the high dose of dextromethorphan to increase locomotor activity, however these results are not significant ($F(1,30)=2.163$, $p>0.1$, $n=15$). **B)** Treatment with the low dose of lorcaserin appears interact with the high dose of dextromethorphan, but these results are not significant. Treatment with the high dose of lorcaserin resulted in significant interactions with the high dose of dextromethorphan ($F(1,30)=7.42$, $p<0.01$, $n=15$). Both doses of lorcaserin appeared to reduce locomotor activity with dextromethorphan in a dose-dependent manner, however these results are not significant ($F(4,60)=0.224$, $p>0.5$, $n=15$).

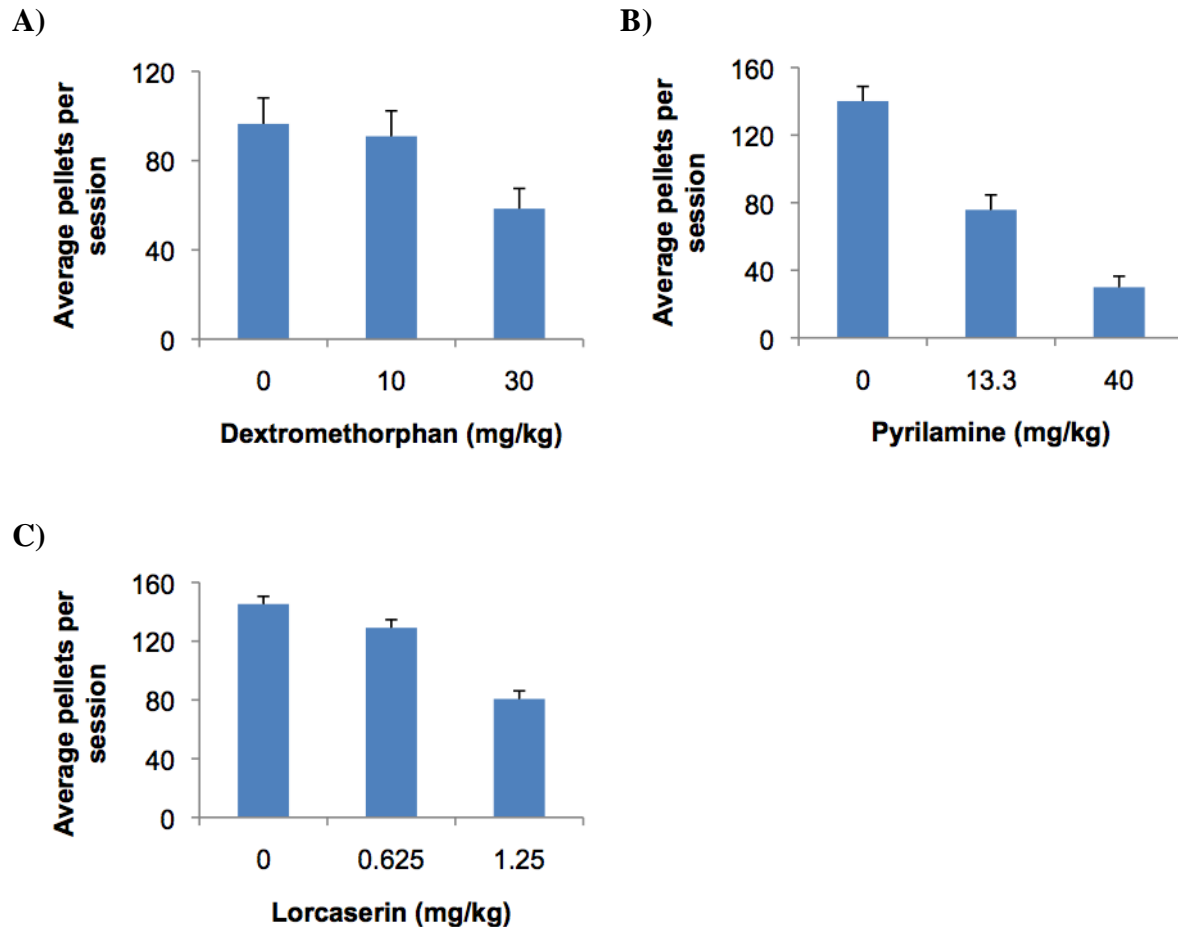


Figure 4: Single Drug Effects on Appetite and Food Self-Administration

Average pellets per session refer to the average number of food pellets delivered per 30-minute self-administration session with each dose combination. **A)** In the dextromethorphan-pyrilamine study, treatment with dextromethorphan significantly decreased food self-administration in a dose-dependent manner ($F(2,22)=58.094$, $p<0.0005$, $n=11$). The high dose of dextromethorphan significantly decreased food self-administration relative to saline injection ($F(1,22)=8.287$, $p<0.01$, $n=11$). The low dose did not significantly decrease food self-administration relative to saline injection ($F(1,22)=0.450$, $p>0.5$, $n=11$). Identical trends for effects of dextromethorphan alone on food self-administration were observed in the dextromethorphan-lorcaserin study. **B)** Treatment with pyrilamine did significantly decrease food self-administration in a dose-dependent manner ($F(2,22)=58.094$, $p<0.0005$, $n=11$). Both the high and low doses of pyrilamine resulted in a significant decrease in food self-administration relative to saline injection (High dose: $F(1,22)=77.425$, $p<0.0005$, $n=11$) (Low dose: $F(1,22)=10.677$, $p<0.005$, $n=11$). **C)** Treatment with lorcaserin did significantly decrease food self-administration in a dose-dependent manner ($F(2,30)=42.889$, $p<0.0001$, $n=15$). Both the high and low doses of lorcaserin resulted in a significant decrease in food self-administration relative to saline injection (High dose: $F(1,30)=79.192$, $p<0.0001$, $n=15$) (Low dose: $F(1,30)=4.959$, $p<0.05$, $n=15$).

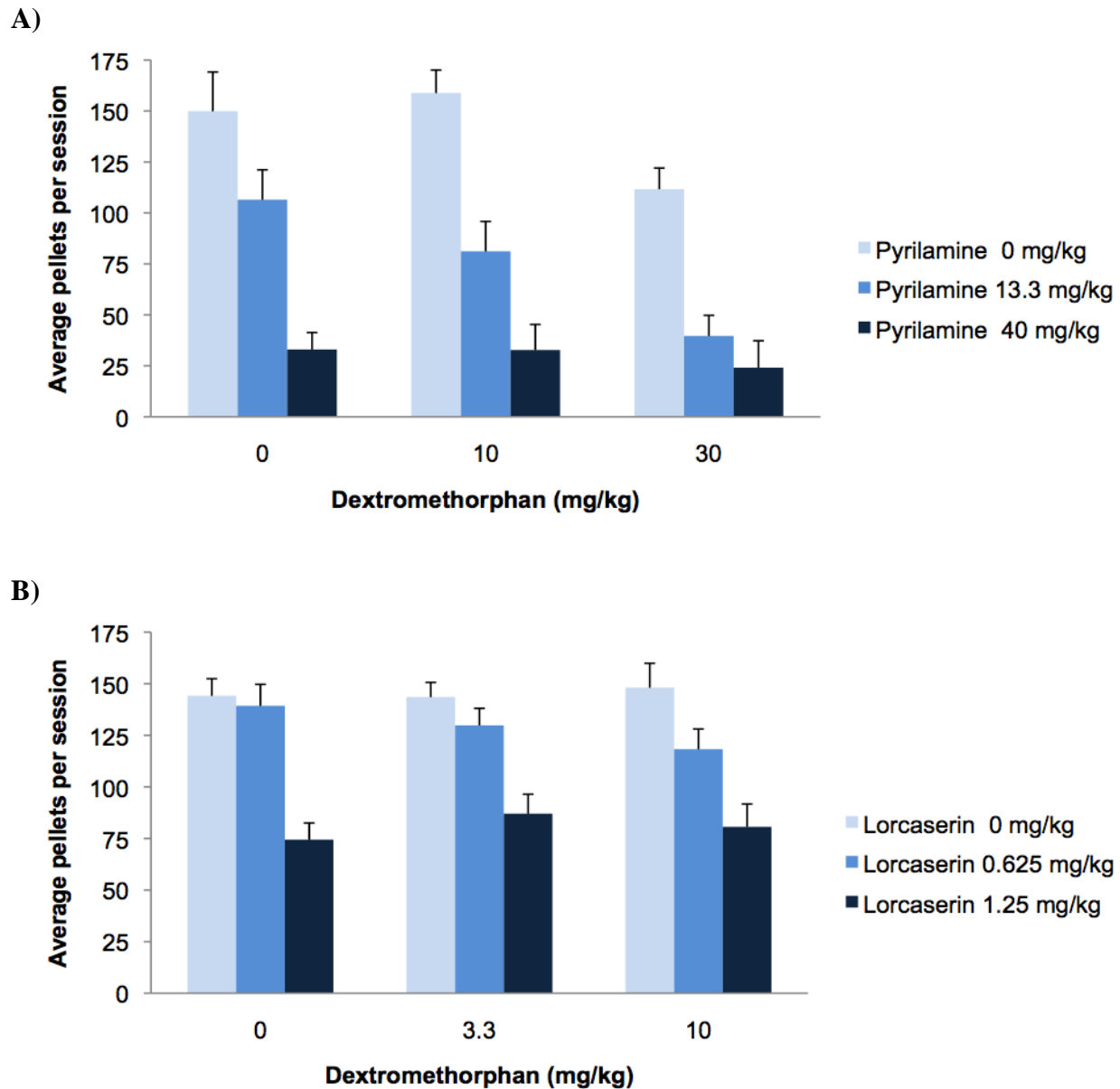


Figure 5: Drug Interactions on Food Self-Administration

Average pellets per session refer to the average number of food pellets delivered per 30-minute self-administration session with each dose combination. **A)** Treatment with the low dose of pyrilamine did have significant interactions with both the low and high doses of dextromethorphan, resulting in greater reductions in food self-administration than with either dose alone (Low dose: $F(1,22)=34.166$, $p<0.0005$, $n=11$) (High dose: $F(1,22)=90.042$, $p<0.0005$, $n=11$). Treatment with the high dose of pyrilamine also had significant interactions with both the low and high doses of dextromethorphan (Low dose: $F(1,22)=29.363$, $p<0.0005$, $n=11$) (High dose: $F(1,22)=43.448$, $p<0.0005$, $n=11$). **B)** No significant interactions were observed between dextromethorphan and lorcaserin in food self-administration. Dextromethorphan and lorcaserin did not interact in a dose-dependent manner ($F(4,60)=0.895$, $p>0.1$, $n=15$).

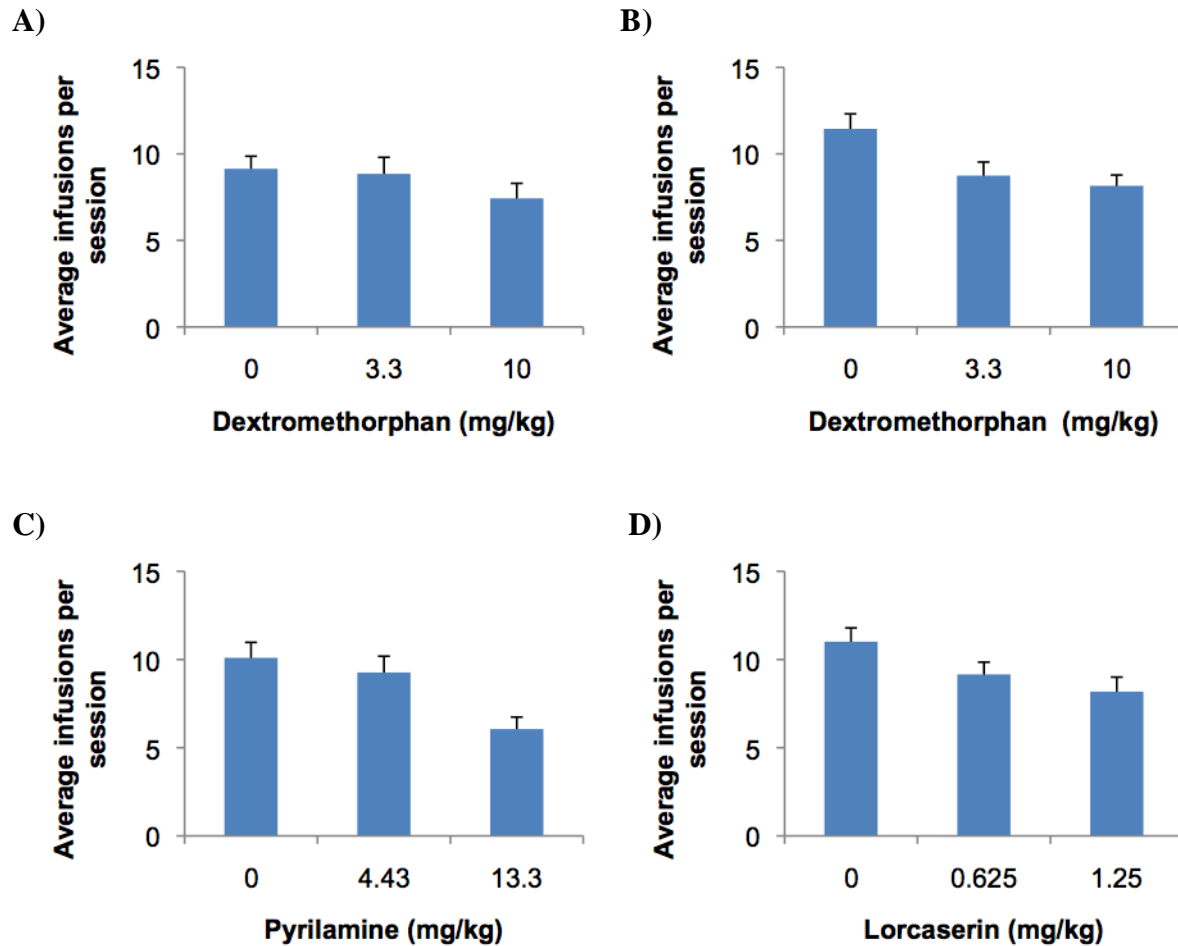


Figure 6: Single Drug Effects on Nicotine Self-Administration

Average infusions per session refer to the average number of nicotine infusions delivered per 1-hour self-administration session with each dose combination. **A)** In the dextromethorphan-pyrilamine study, treatment with dextromethorphan alone was not shown to significantly decrease nicotine self-administration in a dose-dependent manner ($F(2,22)=2.842$, $p>0.05$, $n=11$). **B)** In the dextromethorphan-lorcaserin study, treatment with dextromethorphan was shown to significantly decrease nicotine self-administration in a dose-dependent manner ($F(2,28)=18.539$, $p<0.0001$, $n=14$). Both the high and low doses resulted in a significant decrease in nicotine self-administration relative to saline injection (Low dose: $F(1,28)=21.972$, $p<0.0001$, $n=14$) (High dose: $F(1,28)=32.601$, $p<0.0001$, $n=14$). **C)** Treatment with pyrilamine significantly decreased nicotine self-administration in a dose-dependent manner ($F(2,22)=12.605$, $p<0.001$, $n=11$). The high dose of pyrilamine significantly decreased nicotine self-administration relative to saline injection ($F(1,22)=22.608$, $p<0.0005$, $n=11$). The low dose did not significantly decrease nicotine self-administration relative to saline injection ($F(1,22)=0.961$, $p>0.1$, $n=11$). **D)** Treatment with lorcaserin ten minutes prior to nicotine self-administration sessions significantly decreased nicotine self-administration in a dose-dependent manner ($F(2,28)=4.143$, $p<0.05$, $n=14$). The high dose of lorcaserin significantly decreased nicotine self-administration relative to saline injection ($F(1,28)=8.029$, $p<0.01$, $n=14$). The low dose did not significantly decrease nicotine self-administration relative to saline injection ($F(1,28)=3.443$, $p>0.05$, $n=14$).

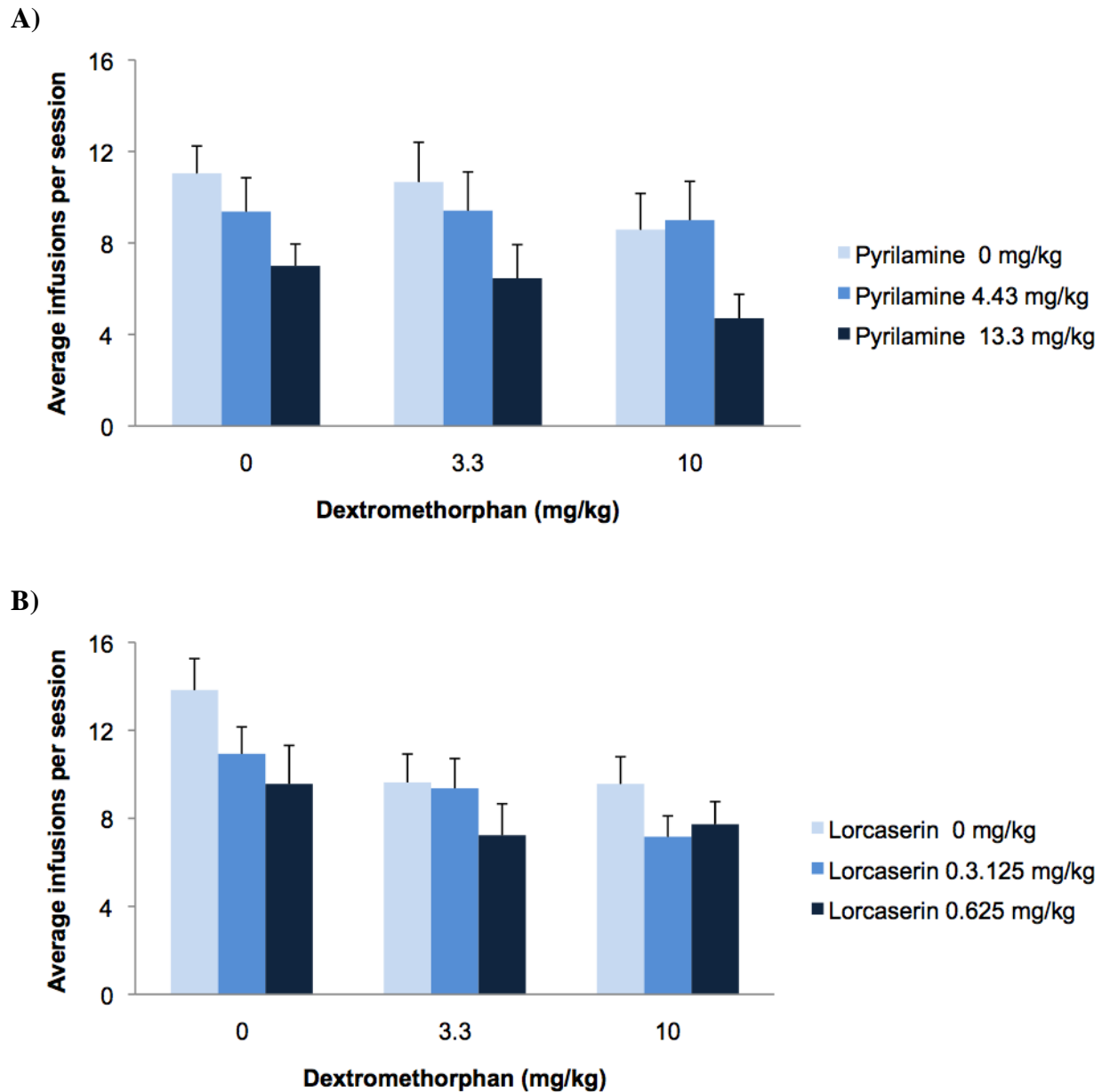


Figure 7: Dextromethorphan and Lorcaserin Interactions in Nicotine Self-Administration

Average infusions per session refer to the average number of nicotine infusions delivered per 1-hour self-administration session with each dose combination. **A)** No significant interactions were observed between dextromethorphan and pyrilamine that resulted in greater reductions in nicotine self-administration compared to either drug alone. **B)** Treatment with the high dose of lorcaserin significantly interacted with the low dose of dextromethorphan to reduce nicotine self-administration by a greater margin than either dose alone ($F(1,56)=5.764$, $p<0.05$, $n=14$). Treatment with the low dose of lorcaserin also significantly interacted with the high dose of dextromethorphan ($F(1,56)=14.198$, $p<0.0005$, $n=14$).

Discussion

Locomotor Activity

The results of the locomotor activity trials suggest that the 30 mg/kg dose of dextromethorphan, the 13.3 and 40 mg/kg doses of pyrillamine, and the 0.625 and 1.25 mg/kg doses of lorcaserin cause significantly reduced activity levels. Since potential sedating effects are considered problematic for a potential nicotine addiction treatment and should be avoided, the investigation of the effects of lower doses of these drugs on locomotor activity may be in order.

Food Self-Administration

Increased appetite and weight gain are common side effects associated with most traditional smoking cessation therapies, and novel treatment options should look to mitigate these side effects. With this in mind, the significant decreases in food self-administration observed with dextromethorphan, pyrillamine, and lorcaserin and the interactions between dextromethorphan and pyrillamine are promising for a potential smoking cessation drug. More work should be done to determine if these effects on food self-administration attenuate with longer exposure.

Dextromethorphan and Pyrillamine Interactions

In the dextromethorphan-pyrillamine study, no significant interactions were observed between the two drugs in reducing nicotine self-administration. As has been demonstrated by our laboratory in previous studies, pyrillamine did significantly reduce nicotine self-administration in a dose-dependent manner when compared to saline injection (Levin et al 2011). The results of this study, however, do not demonstrate any statistically significant reduction in nicotine self-administration with dextromethorphan at any dose, contrary to the results observed in Glick et. al. 2001. This may be the result of desensitization following administration of the higher 30

mg/kg dose in locomotor and pellet sessions, and the subsequent reduction of the maximum dose to 10 mg/kg in nicotine sessions. It is also possible that the reductive effects of dextromethorphan on nicotine self-administration observed in the Glick study were the result of sedation, since no locomotor activity or comparable tests were performed. A study using a maximum dextromethorphan dose of 10 mg/kg for locomotor, pellet, and nicotine sessions should be conducted to determine if the 10mg/kg dose of dextromethorphan can be effective at reducing self-administration over a long time frame without causing sedative effects.

Dextromethorphan and Lorcaserin Interactions

In the dextromethorphan-lorcaserin study, however, dextromethorphan was observed to significantly reduce nicotine self-administration in a dose-dependent manner, in contrast to the results observed in the dextromethorphan-pyrilamine study but in accordance with the results seen in Glick et al 2001. This conflicting data suggests that the results observed in the dextromethorphan-pyrilamine study may be anomalous, and that perhaps co-administration with pyrilamine results in desensitization to the nicotine self-administration reduction generally caused by dextromethorphan. Further research is required to characterize this potential relationship between dextromethorphan and pyrilamine and confirm the attenuating effects of dextromethorphan on nicotine self-administration.

Additionally, further inquiry into the use of dextromethorphan in other drug addiction treatments is warranted as previous studies have shown that dextromethorphan may be useful in the treatment of opioid and methamphetamine addiction (Glick et al 2001) and cocaine addiction (Pulvirenti et al 1997). It should be recognized, however, that dextromethorphan has the potential for recreational abuse as a dissociative hallucinogen. Any drug addiction treatment containing dextromethorphan should not simply substitute one addictive drug for another. Future studies

should investigate if rats will self-administer dextromethorphan to evaluate its potential for addiction, and new applications for this drug should be carefully considered.

The dextromethorphan-lorcaserin study also corroborates the results of Higgins et. al. in showing that lorcaserin reduces nicotine self-administration in a dose-dependent manner (2012). Furthermore, significant interactions were observed between the 0.625 mg/kg lorcaserin dose and the 3.3 mg/kg dextromethorphan dose and between the 0.3125 mg/kg lorcaserin dose and the 10 mg/kg dextromethorphan dose. While these results are exciting and warrant further investigation into a combination therapy of dextromethorphan and lorcaserin as a novel treatment for smoking cessation and nicotine addiction, first more work must be done to determine the relationship between these two drugs and why dose-dependent reductive interactions are not observed. Future research should also focus on the neurological foundations of interactions between glutamate antagonism and serotonin agonism in the reward pathway as suggested by the observed dextromethorphan-lorcaserin interactions.

Future Interaction Studies

Looking forward, the results of these studies suggest that dextromethorphan, pyrillamine, and lorcaserin all have great promise for potential use in treating nicotine addiction. Future work should explore the interactions of pyrillamine and lorcaserin. Additional interaction studies should be conducted on dextromethorphan with a number of other agonists and antagonists to neurotransmitter molecules in the reward pathway and in related neurological systems. The nicotinic acetylcholine receptor antagonist mecamylamine (Zevin et al 2000) and alpha-4 beta-2 partial agonist sazetidine-A have (Levin et al 2010) also been previously shown to significantly decrease nicotine self-administration independently, and are thus ideal candidates for future interaction studies.

Acknowledgements

I would like to acknowledge everyone in the Levin Lab for their support, especially Dr. Edward Levin for his leadership and valuable discussion, and Corinne Wells and Susan Slade for their early instruction and continued assistance and mentorship during this project. I would also like to thank Dr. Vikas Bhandawat for his guidance and support through writing this thesis. Finally, I would like to acknowledge the financial support of the National Institute on Drug Abuse, which funded this research through NIDA P50 Grant DA027840.

References

- Bubar MJ and Cunningham KA. Serotonin 5-HT_{2A} and 5-HT_{2C} receptors as potential targets for modulation of psychostimulant use and dependence. *Curr. Top. Med. Chem.* 2006; 6(18): 1971-1985.
- Cami J, Farre M. Mechanisms of disease: Drug addiction. *N. Engl. J. Med.* 2003; 349: 975-986.
- Cunningham KA, Fox RG, Anastasio NC, Bubar MJ, Stutz SJ, Moeller FG. Selective serotonin 5-HT_{2C} receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-salience value of cocaine vs sucrose-associated cues. *Neuropharmacology.* 2011; 61: 513–523.
- Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. *Neuron.* 1996; 16: 905-908.
- Dani JA. Overview of nicotinic receptors and their roles in the central nervous system. *Biol. Psychiatr.* 2001; 49(3): 166-174.
- Giovino GA. The tobacco epidemic in the United States. *Am. J. Prev. Med.* 2007; 33(6S): S318–S326.
- Glick SD, Maisonneuve IM, Dickinson HA, Kitchen BA. Comparative effects of dextromethorphan and dextrorphan on morphine, methamphetamine, and nicotine self-administration in rats. *Eur. J. Pharmacol.* 2001; 422(1-3): 87-90.
- Hernandez SC, Bertolino M, Xiao Y, Pringle KE, Caruso FS, Kellar KJ. Dextromethorphan and its metabolite dextrorphan block $\alpha 3\beta 4$ neuronal nicotinic receptors. *J. Pharmacol. Exp. Ther.* 2000; 293: 962-967.

Higgins GA, Silenieks LB, Rosmann A, Rizos Z, Noble K, Soko AD, Fletcher PJ. The 5-HT_{2C} Receptor Agonist Lorcaserin Reduces Nicotine Self-Administration, Discrimination, and Reinstatement: Relationship to Feeding Behavior and Impulse Control.

Neuropsychopharmacology. 2012; 37(5): 1177-1191.

Hughes JR, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction*. 2004; 99: 29-38.

Levin ED, Rezvani AH. Nicotinic interactions with antipsychotic drugs, models of schizophrenia and impacts on cognitive function. *Biochem. Pharmacol*. 2007; 74(8): 1182-1191.

Levin ED, Slade S, Johnson M, Petro A, Horton K, Williams P, Rezvani AH, Rose JE. Ketanserin, a 5-HT₂ receptor antagonist, decreases nicotine self-administration in rats. *Eur. J. Pharmacol*. 2008; 600(1-3): 93-97.

Levin ED, Rezvani AH, Xiao Y, Slade S, Cauley M, Wells C, Hampton D, Petro A, Rose JE, Brown ML, Paige MA, McDowell BE, Kellar KJ. Sazetidine-A, a Selective alpha-4 beta-2 Nicotinic Receptor Desensitizing Agent and Partial Agonist, Reduces Nicotine Self-Administration in Rats. *J. Pharmacol. Exp. Ther*. 2010; 332: 933-939.

Levin ED, Slade S, Wells C, Pruitt M, Cousins V, Cauley M, Petro A, Hampton D, Rose J. Histamine H₁ antagonist treatment with pyrilamine reduces nicotine self-administration in rats. *Eur. J. Pharmacol*. 2011; 650(1): 256-260.

McEvoy J, Freudenreich O, McGee M, Vanderzwaag C, Levin E, Rose J. Clozapine decreases smoking in patients with chronic schizophrenia. *Biol. Psychiatr*. 1995; 37: 550-552.

Norton S, Culver B, Mullenix P. Development of nocturnal behavior in albino rats. *Behav. Biol*. 1975; 15: 317-331.

Pulvirenti L, Balducci C, Koob GF. Dextromethorphan reduces intravenous cocaine self-administration in the rat. *Eur. J. Pharmacol.* 1997; 321: 279–283.

Rice ME, Cragg SJ. Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neurosci.* 2004; 7(6): 583-584.

Ruppert PH, Dean KF, Reiter LW. Development of locomotor activity of rat pups in figure-eight mazes. *Dev. Psychobiol.* 1985; 18(3): 247-260.

Timofeeva OA, Roegge CS, Seidler FJ, Slotkin TA, Levin ED. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. *Neurotoxicol. Teratol.* 2008; 30: 38-45.

Zevin S, Jacob P, Benowitz NL. Nicotine-mecamylamine interactions. *Clin. Pharmacol. Ther.* 2000; 68: 58-66.