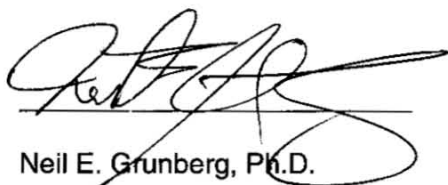


APPROVAL SHEET

Title of Thesis: "Behavioral effects of enrichment and nicotine in
female Sprague Dawley rats"

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May, 2009

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Report Documentation Page			Form Approved OMB No. 0704-0188		
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE MAY 2009		2. REPORT TYPE		3. DATES COVERED 00-00-2009 to 00-00-2009	
4. TITLE AND SUBTITLE Behavioral Effects Of Enrichment And Nicotine In female Sprague Dawley Rats			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Uniformed Services University of the Health Sciences,4301 Jones Bridge Rd,Bethesda,MD,20814			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Three experiments examined effects of environmental enrichment and nicotine on body weight, food consumption, and activity in 52 female Sprague Dawley rats. Rats were housed in physically, socially, or super (social and physical) enriched or non-enriched environments. Half the animals received nicotine for 14 days. Rats in the super-enriched group had: attenuated body weight gain, increased home cage activity, and decreased open-field locomotor activity. Enrichment did not appear to affect voluntary exercise. Rats in the nicotine group, compared with the saline group, had decreased body weight in all housing conditions and increased voluntary exercise only in the physicallyenriched environment. Overall, the cessation of nicotine increased body weight and food consumption. These findings and their implications for women?s health are discussed.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 135	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

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ABSTRACT

Title of Thesis: "Behavioral effects of enrichment and nicotine in female Sprague Dawley rats"

Author: Cynthia A. Rose, Master of Science, 2009

Thesis directed by: Neil E. Grunberg, Ph.D., Professor
Department of Medical and Clinical Psychology

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Behavioral Effects of Enrichment and Nicotine
in Female Sprague Dawley Rats

by

Cynthia A. Rose

Master's Thesis submitted to the Faculty of the
Department of Medical and Clinical Psychology
Graduate Program of the Uniformed Services University
of the Health Sciences in partial fulfillment
of the requirements for the degree of
Master of Science, 2009

ACKNOWLEDGEMENTS

I am so grateful to the many people who have helped me with this process. From the very moment I joined the Grunberg lab, I was graciously taken under the wings of all my labmates (Christie Oates Simpson-McKenzie, Michael Perry, Sarah Shafer Berger, Kristen Hamilton, Amy Starosciak, and Stephanie Long). My labmates have not only taught me about research, but they have truly become my friends. I have never met a more supportive group of people that I can trust and count on, both educationally and personally.

I am even more grateful to my advisor Neil E. Grunberg. He has pushed me to limits that I did not know I was capable and has guided me to grow as a person in many ways. I am thankful for the valuable input and time devoted to my master's thesis by my committee members, Michael Feuerstein and Andrew Waters. They have taught me to think both as a researcher and a clinician. My family and friends- I would not be here right now if it were not for their constant support. All the time (and phone calls!) kept me motivated and helped me with prioritizing. Thanks for sticking by my side (even when I made it difficult!).

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Overview

Currently, the field of women's health is evolving and growing, and the need for research examining women's health is greater than ever in order to promote health equity and discover sex-specific approaches and interventions. Tobacco use, food consumption, and physical activity all are behaviors that affect health. Tobacco use, physical activity, food consumption, and environment influence body weight. The present experiments were designed to examine all of these variables in female subjects. More specifically, three laboratory experiments were conducted to examine effects of several types of environmental enrichment on activity, food consumption, and body weight; environmental enrichment and nicotine administration on body weight, food consumption, and activity; and environmental enrichment and the cessation of nicotine on body weight, food consumption, and activity in females. Rats were the subjects to control variables under study and to administer nicotine to drug-naïve subjects—a procedure that would be difficult and, perhaps, unethical in humans.

Importance of the Topic

Currently, the leading causes of mortality and morbidity in the United States are cigarette smoking and obesity. Tobacco kills 440,000 people per year in the United States, accounting for roughly one out of every five deaths, and kills over five million people worldwide each year. It is estimated that annual deaths worldwide from tobacco will reach 10 million by the year 2020 (CDC, 2006). Cigarette smoking is the single most important factor contributing to premature

mortality in the United States. The overall premature mortality ratio for all smokers of cigarettes is about 2.0 compared to nonsmokers, which means that smokers have a 100% greater chance than a nonsmoker to die prematurely (CDC, 2006). Cigarette smokers have a higher incidence of all illnesses. It is estimated that 20.8% of all adults (45.3 million people) smoke cigarettes in the U.S. (CDC, 2007). To address the impact that smoking has on public health, it is important to understand nicotine. Nicotine is the addictive component in tobacco products that has the potential to increase smoking and exposure to the toxic chemicals found when smoking (USDHHS, 1988). While nicotine in tobacco has many negative health consequences, nicotine decreases body weight and food consumption (Grunberg, Bowen, & Morse, 1984; Grunberg, 1985; Grunberg, Bowen, & Winders, 1986; Grunberg, Winders, & Popp, 1987; Grunberg, 1990; French & Jeffrey, 1998), which is relevant to the present experiments.

The United States is in the midst of an obesity epidemic. Obesity refers to an excess amount of body fat. Obesity is the fastest growing cause of illness and death in the United States and is second only to cigarette smoking as a leading cause of preventable death in the United States (CDC, 2008). Obesity results in 300,000 excess deaths each year and had an economic cost in the U.S. of about \$117 billion dollars in 2000. Treating obesity-related diseases constitutes almost 10% of health care dollars (CDC, 2008). To address the impact obesity or excessive overweight is having on public health it is important to understand the factors that influence body weight. Body weight is influenced by energy input and expenditure. Therefore, it is important that research

consider food consumption (energy input) and activity and exercise (energy expenditure) when studying obesity and excessive overweight, which is one of the purposes of the present experiments.

Cigarette smoking, food consumption, and activity are modifiable behaviors. Important factors that must be examined when dealing with human behaviors are individual differences and the environment. In order to understand someone's behaviors, both the person (individual differences) and their environment need to be examined because behavior, according to Lewin (1951), is a function of the person and their environment. One important individual difference that research should examine is sex differences. Because sex differences exist, it is important to examine this variable in research because males and females can be similar or different depending on the issue studied. Research should also examine both the physical and social environments of individuals when studying cigarette smoking and obesity or excessive overweight, as there may be differences between males and females. The present experiments examine these factors in females.

Recently, Long (2008) examined all of these variables in male rats. The purpose of the present experiments was to determine if these factors have similar or different effects in female rats. As background for the present experiments, relevant information about obesity and body weight, physical activity, tobacco use, and environmental enrichment are presented. Then, each of the three experiments is presented, including hypotheses, methods, results,

and discussion. A general discussion follows with implications, limitations, and future directions.

Obesity/Overweight

Mokdad, Marks, Stroup, and Gerberding (2004) suggest that obesity/excessive overweight may soon overtake tobacco as the leading cause of death in the United States. Approximately 66.5% or 129 million Americans are classified as either overweight or obese, with 32.2% being obese. About 17.1% of children and adolescents are overweight, and the prevalence of overweight children and adolescents has tripled in the past two decades (CDC, 2008). Being overweight or obese in childhood and adolescence poses a significant health risk because overweight and obesity during early life is a good predictor of being overweight and obese in adulthood (Dietz, 1998). In adulthood, women are more likely to be obese than men, with 33.2% of women versus 31.1% of men being classified as obese (CDC, 2008). Obesity is one of the most significant health problem facing American women (Albu et al., 1997; USDHHS, 2009), which is why the current research is examining body weight in females.

Many factors affect obesity. Genetics play a role in obesity where holding energy output constant, certain people are more likely to gain weight when overeating, or when holding energy intake constant, others lose less weight through excessive exercising (CDC, 2007). According to obese-normal weight twin pair studies, the influence of genetics can be overcome by changing both diet and exercise patterns (CDC, 2007). Metabolism, the amount of energy required to maintain the body at rest, also affects obesity, and metabolism is

affected by genetics, body composition, eating patterns, food restrictions, smoking, activity patterns, and age (CDC, 2007; McCrory, Suen, & Roberts, 2002). Nutrition is a major factor affecting obesity. Energy intake, or food consumption, has increased 15% over the past two decades, where restaurants and fast foods have higher caloric density, higher saturated fats, and larger portion sizes. Physical activity is another factor affecting obesity. The level of energy during work and leisure activities has declined in the U.S. as automation has nearly eliminated the physical demands of many occupational and home activities, and fewer numbers of public schools are requiring physical education classes for youth (Bouchard & Blair, 1999; Lewis, 2007). The environment that surrounds Americans is filled with higher caloric foods and promotes sedentary lifestyle. The psychological aspects of an individual are an important factor affecting obesity, because some individuals eat in response to negative emotions, uncontrollably eat, or eat when they are not hungry (Lowe & Fisher, 1982; Gibson, 2006).

The research on obesity/excessive overweight indicates that there are sex differences, with more women becoming obese and overweight than men. In addition, physical and social environments promote an increase in energy input (food consumption) and a decrease in energy expenditure (activity) that is influencing this obesity epidemic, so it is critical to examine these factors together.

Exercise on Health Outcomes

Physical activity is any bodily movement that results in energy expenditure beyond resting energy expenditure (USDHHS, 1999). Exercise is a subset of physical activity that is planned, structured, repetitive, purposeful, and where the objective is improvement or maintenance of physical fitness (USDHHS, 1999).

Nearly half of young people aged 12-21 are not vigorously active on a regular basis, and physical activity declines dramatically with age during adolescence (USDHHS, 1999). Female adolescents are much less physically active than male adolescents (Trost et al., 2002). Inactivity continues to decrease with age (Trost et al., 2002). More than 60% of adults do not achieve the recommended amount of regular physical activity, which is 30 minutes of moderate intensity activity five days per week or 20 minutes of intense activity three times a week (CDC, 2007). Inactivity is more common among women, where only 19.5% participate in regular activity (Collins et al., 1999; USDHHS, 1999) and 26.2% report no leisure physical activity (CDC, 2007).

Physical activity, especially exercise, is important because it has many physical and mental health benefits (Manning & Fusilier, 1999; USDHHS, 1999; CDC, 2007). Exercise reduces the risk of cardiovascular disease and high blood pressure, prevents atherosclerosis, enhances immune function, controls blood glucose, and increases the number and efficiency of white blood cells (USDHHS, 1999). Exercise can help prevent depression and is as effective as an antidepressant medication in the treatment of depression (Dubbert, 2002). Exercise also improves quality of life (Brown et al., 2003).

Physical activity is an important public health concern, especially among females because females tend to have lower levels of physical activity. The present experiments focus on variables that affect physical activity in females.

Exercise on Obesity

Energy balance is composed of energy intake and energy expenditure (Spiegelman & Flier, 2001). The balance influences body weight. Energy intake and energy expenditure must be equivalent or weight will be either gained or lost (Spiegelman & Flier, 2001). Positive energy balance occurs when energy intake (excess caloric intake) exceeds energy expenditure, resulting in weight gain (Hill, Wyatt, Reed, & Peters, 2003). Negative energy balance occurs when energy intake does not exceed energy expenditure, resulting in weight loss (Hill et al., 2003). Exercise contributes to the energy expenditure portion of the equation (Hill et al., 2003).

Exercise is important for people with obesity, not only for weight loss, but also for health-related outcomes. Myers et al. (2002) found that the relative risk of death for patients who were obese was linked to their level of fitness. Although fitness in obese patients did not extinguish the risk of death, the higher the level of fitness, the lower the relative risk of death a person with obesity faced. It is important to continue to study factors that affect exercise in female populations because research has already indicated that females exercise less and are more likely to be obese/overweight than males.

Cigarette Smoking

Cigarette smoking is another behavior that has great effects on women's health. Cigarette smoking has negative health consequences and causes more deaths each year than all deaths from HIV, illegal drug use, alcohol use, motor vehicle injuries, suicides, and homicides combined. Cigarette smoking is more common among men than women with 23.9% of males and 18.1% of females being smokers (CDC, 2006). Historically, males had greater disease and mortality ratios than females due to cigarette smoking, but now men and women have similar rates. The mortality risk is similar particularly after women have passed menopause. Since 1987, lung cancer (a major cause of mortality caused by cigarette smoking) had surpassed breast cancer as the leading cause of cancer-related deaths in women. Cigarette smoking is particularly dangerous for women with regard to its effects on pregnancy. Cigarette smoking leads to an increased amount of infertility (ACOG, 1993), and births from women who smoke are two times more likely to have low birth weight (ACLBWSG, 1990; Hellerstedt, Himes, Story, Alton, & Edwards, 1997).

There are many factors that reinforce nicotine, the addictive component of tobacco, self-administration. Some of these factors include appetite and body weight control, increased attention and information processing, mood regulation, relief of boredom, increase relaxation, social contexts, and coping with stress (Koob & Le Moal, 2006; USDHHS, 1988). Many individuals smoke with weight-loss related intentions; however, current smoking also is related to obesity-

promoting behaviors such as diminished use of exercise facilities (Carroll et al., 2006).

The effects of nicotine have clear gender differences (Grunberg, Winders, & Wewers, 1991; Perkins, Donny, & Caggiula, 1999). Women appear to respond more than men to non-nicotine effects of smoking, such as smoking in social gatherings (Perkins, Sexton, & Di Marco, 1996), which is why enriched environments in addition to nicotine use should be examined together in females. Women also are more likely than males to smoke for weight management reasons (Crisp, Sedwick, Halek, Joughin, & Humphrey, 1999; Kristeller & Johnson, 1997). Grunberg and colleagues (1986) found that female rats showed greater sensitivity to higher doses of nicotine with regard to body weight than male rats, where female rats administered a high dose of nicotine (12 mg nicotine/kg/day) had lower body weights than before nicotine administration, which was not found in male rats. Nicotine not only attenuated body weight gain, but it actually decreased weight in females.

The research literature indicates that the effects of nicotine have clear sex differences. Females have a greater reaction to nicotine in terms of body weight and food consumption (Grunberg, Bowen, & Winders, 1986; Grunberg, Winders, & Popp, 1987; Winders & Grunberg, 1989; Grunberg, 1992), which is important when taking into consideration the prevalence of obesity/excessive overweight in females and the decreased amount of activity in females. Also, environments appear to influence the behavior of nicotine self-administration (e.g., smoking more in social gatherings [Perkins, Sexton, & DiMarco, 1996]). Therefore, it is

important to examine effects of both nicotine and environmental enrichment in females.

Environmental Enrichment

As mentioned previously, behaviors are a result of the interaction between a person and their environment (Lewin, 1951). Environmental enrichment, interacting with environmental stimuli, has enduring effects on a variety of factors, including healthy development in both humans and animals.

Environmental enrichment also can promote healthy behaviors, including physical activity (Tomchesson, 2006; Shafer, 2006; Long 2008). The earliest account of the modern day perception of environmental enrichment came from the observations of Charles Darwin in the late 19th century. Darwin (1875) observed that different environments affected the brain sizes in rabbits. Hebb (1947) noticed learning differences between rats that were raised in the laboratory versus laboratory rats that he took home as pets, where rats that were exposed to “enriched environments” as pets subsequently performed better on tasks. Rosenzweig (1966) developed the environmental enrichment paradigm 20 years after Hebb’s work. Rosenzweig’s paradigm included a model of social enrichment that consisted of animals being group housed and a model of physical enrichment that included placing objects or toys in animals’ home cages. The enriched environments used in the present experiments were modeled after Rosenzweig’s paradigm.

The basis for Rosenzweig’s paradigm was that physical and social components of an environment can influence the biology of organisms.

Environmental enrichment can enhance learning, memory, and improve information processing in organisms (Smith, 1972; Gardner, Boitano, Manvico, & D'Amico, 1975; Daniel, Roberts, & Dohanick, 1999; Van Praag, Kempermann, & Gaage, 1999; Varty, Paulus, Braff, & Geyer, 2000; Woodcock & Richardson, 2000).

Physical and social components of an environment also can influence the behavior of organisms. There are several lines of research that report that rats exhibit more complex behaviors in enriched environments (Mohammad et al., 1993; Pham et al., 1999; Kobayashi, Ohashi, & Ando, 2002). Animals in enriched environments show quicker adaptation in the acoustic startle response paradigm (Swerdlow, Caine, Braff, & Geyer, 1992). Animals in enriched environments also show a reduction in emotionality, where animals with reduced sensory stimulation (lacking enriched environments) showed hyperemotionality (Haywood & Tapp, 1966). Wemelsfelder, Haskell, Mendl, Calvert, and Alistair (2000) also found that pigs in enriched environments had more diverse behaviors.

Environmental enrichment has robust, positive consequences on organisms. These positive consequences occur at both the biological and behavioral levels. Animals raised in non-enriched environments or isolation have shown cognitive and behavioral disruptions. Because eating behaviors and cigarette smoking affect both the biology and behavior of organisms, as does environmental enrichment, environmental enrichment may have profound effects

on obesity/excessive overweight and cigarette smoking (or nicotine's actions), which is why the present experiments examined these factors together.

Environmental Enrichment on Body Weight, Food Consumption, and Activity

Enriched environments have some reported effects on energy intake and energy expenditure, but the effects are not clear. Brown and Grunberg (1996) reported that enriched environments slightly decreased food consumption. Tomchesson (2004) found that environmental enrichment decreased food consumption of standard rat chow, and it also decreased consumption of high fat foods (Oreos® and potato chips) in male rats (Tomchesson, 2006). Tomchesson (2004, 2006) also found that environmental enrichment attenuated weight gain. Environmental enrichment also has been shown to decrease open field activity in male rats (Elliott & Grunberg, 2005; Shafer, 2006; Tomchesson, 2006), which seems to be influenced by habituation of enriched animals to novel environments. At the same time, environmental enrichment has been shown to increase home cage activity especially when rats were in larger cages (Tomchesson, 2006), but the mechanisms for this increase are unclear. There were four proposed possibilities for this increase in home cage activity: (1) enrichment provides more opportunity and room to engage in activity, (2) enrichment provides an opportunity for social interaction to engage in more playful behaviors between rats, (3) enrichment provides a more novel environment with more toys and area to explore and encourage the natural instincts of rats for foraging and exploration, and (4) enrichment causes biological

changes, such as heart morphology that may impact physical fitness of an organism (Tomchesson, 2006; Shafer, 2006). While these mechanisms remain unclear, it is critical to see if environmental enrichment has the same effects in female rats.

Enrichment and exercise each result in beneficial effects in rats. Both promote central neuronal plasticity and decrease neurodegenerative diseases (Fernandez-Teruel et al., 2002; Johansson, 2003; Klein, Jones, & Schallert, 2003; Kramer, Beatty, Plowey, & Waldrop, 2002; Sutoo & Akiyama, 2003; Elliott, 2004). Voluntary exercise in animals is comparable to exercise habits in humans (Eikelboom, 1999; Sherwin, 1998). When given running wheel access for only 120 minutes per day, the amount of voluntary exercise rats engaged in remained relatively stable (Lattanzio & Eikelboom, 2003). However, external factors, such as enrichment, also play a crucial role in the amount of voluntary exercise in which rodents participate.

There have been several hypotheses posed by the Grunberg research group (Long & Grunberg, personal communication, September 23, 2007) on how housing conditions influence voluntary exercise. The first is that wheel running is a rodent's attempt to compensate for scantiness of natural elements in its environment. However, this hypothesis would not account for the animals in semi-natural or enriched environments that continue to exhibit patterns of exercise behavior. The second is that enriched environments channel the rodents' natural instincts to search and explore environments into exercise. The third is that enrichment in social conditions is a potential motivator for exercise,

but the results have been inconsistent. The motivation for activity remains unclear.

In a study that examined both enrichment and exercise together in male rats, Long (2008) found that housing conditions did not have effects on food consumption, but that environments with both social and physical enrichment attenuated body weight slightly, which was similar to the findings by Tomchesson (2006) and Shafer (2006). Long (2008) found that enriched rats had decreased open field activity that is similar to past studies, indicating quicker habituation to novel environments (Tomchesson, 2004; Elliott & Grunberg, 2005; Tomchesson, 2006; Shafer, 2006). Long (2008) found that physically-enriched male rats exhibited the greatest amount of exercise activity, but rats that received both physical and social enrichment exhibited the least amount of exercise activity compared to rats raised in social environments, both social and physical environments, and rats raised with no enrichment. Long's (2008) results may be applicable to addressing the issues of environment on excessive overweight/obesity behaviors in males. However, because females may have different responses, (e.g., Brown & Grunberg [1995] found that female rats were more stressed when isolated and less stressed when in a socially enriched environment), it may be that enriched environments particularly with a social component may be more beneficial for females than males. Therefore, it is important to examine the effects of different types of enrichments in females.

Effects of Nicotine on Food Consumption, Body Weight, and Activity in Different Housing Conditions

The influence of nicotine on energy intake and expenditure have been well established. Nicotine attenuates weight gain, decreases food consumption, and slightly increases activity (Elliott, Faraday, Phillips, & Grunberg, 2004; Faraday, Scheufele, Rahman, & Grunberg, 1999; Grunberg, Bowen, & Morse, 1984; Grunberg, 1982; Grunberg, 1985; Grunberg & Bowen, 1985; Grunberg, Winders, & Popp, 1987; Saah, Raygada, & Grunberg, 1994; Winders & Grunberg, 1989; 1990). Enriched housing's effects on body weight and food consumption are similar to the effects of nicotine in that enriched environments attenuate weight gain (Long, 2008; Shafer, 2006; Tomchesson, 2006), but effects of housing on activity depend on the type of activity.

Environmental enrichment attenuates the effects of acute and repeated acute nicotine administration on open field activity in male rats (Green, Cain, Thompson, & Bardo, 2003; Elliott & Grunberg, 2005). However, the combined effects of environmental enrichment and chronic administration of nicotine and cessation on body weight, food consumption, and activity have only been examined in one study using male rats (Long, 2008).

Long (2008) reported that environmental enrichment decreased open field activity even with nicotine administration, consistent with previous studies of increased habituation. Chronic nicotine administration increased exercise in all conditions, but interacted with enrichment conditions such that exercise was greatest in the physical enriched environment, followed by the social enriched

environment, the no enriched environment, and the least exercise in the environment with both physical and social enrichment. Home cage activity decreased in the combined social and physical enriched environments even with chronic nicotine administration. Nicotine attenuated body weight gain in all enriched conditions with a slight exaggeration in the physically-enriched environment. Nicotine decreased food consumption in all housing conditions except for the physically-enriched condition. Long's (2008) findings may have implications for males' health with nicotine affecting the physically-enriched males to control body weight and increase exercise, without decreasing food consumption. While these results are interesting, they may not be the same in females. We know from previous research that there are clear sex differences in the effects of nicotine (Grunberg, Winders, & Wewers, 1991; Perkins, Donny, & Caggiula, 1999), with females being more sensitive to the effects of nicotine on body weight and feeding (Grunberg, Bowen, & Winders, 1986). Research examining effects of environmental enrichment and nicotine on food consumption, body weight, and activity, in females is warranted.

Benefits of Animal Models

Previous studies have used animal models to investigate environmental factors' influence on health behaviors. Animal models allow increased experimental control and the ability to conduct experiments that would not be logistically feasible or ethical in human research. Animal models lack unique aspects of the human experience, but the benefits of using an animal model provide a valuable first step.

Overview of the Present Experiments

The present project was designed as a follow up to Long's (2008) study of effects of environmental enrichment and nicotine on food consumption, body weight, and activity of male rats, but examining female rats. This research project included three experiments. Experiment I evaluated the effects of environmental enrichment (no enrichment or isolated [NE], physical enrichment [PE], social enrichment [SE], and both physical and social enrichment [SUPER]) on food consumption, body weight, and activity in female rats. Three separate measurements of activity were measured: home cage activity, movement in open field locomotion chamber, and voluntary activity in exercise wheels. Experiment IIa examined the effects of environmental enrichment and chronic nicotine administration on body weight, food consumption, and activity. Experiment IIb examined the effects on environmental enrichment and nicotine cessation on body weight, food consumption, and activity in female rats. Experiment I was necessary to establish baseline measures of the dependent variables (body weight, food consumption, and activity), as well as to allow the rats to mature to adulthood, thereby giving the rats longer exposure to environmental enrichment. Experiment II introduced one of the key independent variables, nicotine. Experiment II was divided into two parts, a and b, to separate the effects of nicotine administration and cessation. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of

Health Guide for Care and Use of Laboratory Animals (National Institutes of Health [NIH], 1996).

Each experiment is discussed separately, including methods, results, and a brief discussion. After each experiment is reviewed, a general discussion is provided to synthesize the findings from the three experiments, followed by a discussion of clinical implications, limitations, and future directions.

Experiment I

Overview

The present experiment examined effects of different enrichment conditions on food consumption, body weight, and different forms of activity remain in female rats.

Hypotheses

It was hypothesized that enriched housing would:

(1) increase home cage activity, such that the PE > SE = SUPER = NE. This hypothesis was based on the home cage activity findings of Tomchesson (2006) and the social enrichment findings of Elliott (2004) and Shafer (2006), who interpreted home cage activity as reflecting opportunities for movement in the presence of objects and others, and the findings from Brown and Grunberg (1995) who found females to have higher arousal when isolated than when in the presence of others. It was hypothesized that the physically-enriched rats would have a greatest increase in home cage activity because they may have a higher arousal from being housed without another rat, which may lead to an increase in activity, and because they are able to interact with toys. It was hypothesized that

the socially enriched, super enriched, and no enriched rats would have similar home cage activities because the socially enriched and super enriched rats could instigate activity in the other and the no enriched rats could have a higher arousal leading to more activity;

(2) decrease open field activity, such that $SUPER < SE < PE < NE$. This hypothesis was based on the occurrence of habituation over time in the open field locomotor chamber within and between each session in male and female rats (Elliott & Grunberg, 2005; Elliott, 2004; Tomchesson, 2006; Shafer, 2006; Long, 2008).

(3) increase voluntary activity, such that $SUPER < SE < NE < PE$. This hypothesis was based on the findings of Long (2008) on male rats;

(4) attenuate weight gain, such that $SUPER < SE < PE < NE$. This hypothesis was based on the findings of Tomchesson (2006), Shafer (2006), and Long (2008) in male and female rats.

(5) have minimal effects on food consumption, such that $SUPER < SE < PE < NE$. This hypothesis was based on the food consumption findings of Tomchesson (2006) and Shafer (2006) in male and female rats, and the idea that there would be no competition over food in the socially-enriched and super enriched environments because these environments were shown to be calming for females by Brown and Grunberg (1995), although Long (2008) found that super-enriched males had the greater amounts of food consumption, which may be because males being housed together have higher arousal (Brown & Grunberg, 1995) and this arousal may have increased competition over food.

Methods

Subjects

Subjects were 52 female Sprague Dawley rats (Charles River Laboratories). There was one independent variable, housing condition. There were four housing conditions: no enrichment or isolated, physical enrichment, social enrichment, and super enrichment. There were 12 animals in the no enrichment, physical enrichment, and social enrichment conditions; and there were 16 animals in the super enrichment condition. Long's (2008) findings indicated that 12 animals in each condition provided adequate statistical power. A power analysis with Long's (2008) exercise data using nQuery (O'Brien & Muller, 1993) found that 12 animals per condition would provide 95% power with a large effect size of 1.07 for a main effect of housing. However, 16 animals were included in the super-enriched condition to be comparable to the number used by Long (2008) in that housing condition. Rats arrived at 21 days of age (the age at which rats are weaned and separated from their mothers) weighing between 40 and 50 grams. The age and strain of rats picked were the same as the rats used by Long (2008). Sprague Dawley rats were used because they have been the most extensively used strain in behavioral and biological research, are calm, and easy to handle (Harlan Laboratories, 2009). Adolescent rats (approximately 21-55 days old) were used to maximize the developmental impact of environmental enrichment (Douglas, Varlinskaya, & Spear, 2004). On day 2, however, one rat became ill and displayed signs of respiratory distress, and was euthanized by USUHS Laboratory of Animal Medicine staff who found that the rat

suffered from kidney failure, leaving an N=51 and an n=15 in the super-enriched condition.

General Husbandry

All rats in the non-enriched, physically-enriched, and socially-enriched environments were housed in standard polycarbonate cages (42 x 20.5 x 20 cm) and super-enriched rats were housed in three-level galvanized steel cages (76 x 61 x 137 cm) with hardwood chip bedding (Pine-Dri) that were changed two times a week. Subjects had continuous access to food (Harlen Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at approximately 23 degrees C and approximately 40% relative humidity on a 12-hour reversed light/dark cycle in accordance to guidelines set by USUHS IACUC and the NIH Guide for Care and Use of Laboratory Animals (NIH, 1996) Lights were turned off at 0600 hours so that all behavioral measures could be made during the rats active (dark) period.

Independent Variables

Housing Conditions (See Appendix A for pictures)

Non-enriched/Isolated (NE). Animals were single housed in standard polycarbonate rat cages (42 x 20.5 x 20 cm) with a water bottle and food but with no access to enrichment materials.

Physical Enrichment (PE). Animals were single housed in standard polycarbonate rat cages (42 x 20.5 x 20 cm) with water bottle, food, and two toys (e.g., plastic balls, tunnels, plastic igloos) to provide novel physical objects for the

animals. Toys were cleaned and changed two times a week in order to maintain a novel, stimulating environment.

Social Enrichment (SE). Two subjects were housed in standard polycarbonate cages (42 x 20.5 x 20 cm) with a water bottle and food but with no toys.

Super Enrichment (SUPER). Sixteen subjects were housed in two three-level galvanized steel cages (76 x 61 x 137 cm), each with four water bottles, four food cups, and eight toys. Toys were changed two times a week and were distributed with three toys on the top level, three toys on the middle level, and two toys on the bottom level. The cage trays that held the bedding were changed two times a week.

Dependent Variables

Body Weight (BW). Body weight was measured using Sartorius electronic balances programmed to take multiple weighings within a short period of time to account for movement artifacts. Body weight was measured two times a week.

Food Consumption (FC). Food consumption was measured every other day by weighing cage lids with food on top of them or food cups with food in them (SUPER condition). The amount of food consumed was calculated based on the change of weight in food measures on subsequent days. (Therefore, while food consumption was first measured at Day 1, the first calculation for food consumption was at Day 3.) When food was replenished, it was weighed and recorded. For animals that were housed together, total amounts per measurement were divided by the number of animals in a given cage to determine individual amounts of food consumption.

Home Cage Activity (HCA). Home cage activity refers to the animals' activity in its primary living quarters. There were two different types of home cage activity: home cage activity observations on individual rats and home cage activity observations on grouped rat activity by housing condition. Both home cage activity methods occurred while animals were in their normal housing arenas during the dark portion (active period) of the light/dark cycle to maximize animal activity. The procedure of home cage activity observations and recordings were based on Tomchesson (2006).

Home Cage Activity 1-Group Activity (HCA1)

Two experienced, independent observers quietly observed animals and provided a global rating for each experimental housing group. The room was dimly lit with red light. Red light is used during their active period for the rat because albino rats cannot see the red light spectrum. Each observer watched a housing condition for 1-minute and recorded the number of animals engaged in physical activity, and average horizontal, vertical, and center cage locomotion activity on a 7-point Likert format scale, where 1 = none and 7 = all. An average level of effort and amount expended during each activity period also was judged and rated on a 7-point Likert format scale, where 1 = none and 7 = continuous high. In addition, the type of physical activity that each animal engaged in was recorded (e.g., with a physical object, social interaction, combined physical and social interaction, or alone). All behaviors scored were easy to detect. This procedure was repeated two times a week for a total of 10 times throughout this experiment. The order of housing conditions observed was counter-balanced

and the time of observation was varied during the dark cycle of each observation day. The procedure was based on Tomchesson (2006). (See Appendix B for a copy of the HCA rating sheet.) Experimenters were trained in this method by experienced lab members who had previously used the HCA rating sheet. During training, the experienced lab members explained the purpose and procedure. The trainers and trainees then practiced the procedure until the ratings were consistent. Any differences were discussed by the trainers and trainees until an agreement was reached. After approximately eight practice sessions, trainees were proficient in this measurement procedure.

Home Cage Activity 2- Individual Rat Activity (HCA2)

Three previously trained observers quietly observed each animal in home cages, while the room was dimly lit with red light. Each observer watched each animal for 3 minutes and recorded overall activity on a 7-point Likert format scale, where 1 = none and 7 = constant high. The order of rats observed and time of observation was balanced. All behaviors recorded and scored were easy to detect. This procedure was repeated once a week for a total of five times throughout this experiment. This procedure was based on Tomchesson (2006) and was modified by Simpson-Mackenzie (2008). (See Appendix C for a copy of the HCA rating sheet.)

Open Field Locomotion (OF). OF was assessed once every other week for a total of two times during this experiment. Locomotion was measured using an Omnitech/Accuscan Electronics Digiscan infrared photocell system (Omnitech/Accuscan Electronics, Columbus, OH). One-hour activity

measurements were obtained during animals' active or dark cycle in a dedicated procedure room close to the housing room. The temperature and humidity of this procedure room were similar to the housing room. Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena with a Plexiglas lid with multiple ventilation holes (3.5 cm diameter) placed on top of the arena to prevent escape. A photocell array measured horizontal locomotor activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the floor of the arena. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Data were automatically gathered and transmitted to a computer via an Omnitech Model DCM-I-BBV analyzer in twelve 5-minute bins. The interfaced software measures 21 activity variables, including total distance, horizontal and vertical activity, and center time. Chambers were cleaned between subjects with 35% isopropyl alcohol solution.

Activity Wheel. Activity in exercise wheels was measured every other week for a total of two times during this experiment. Running wheel activity was measured using Med Associates (St. Albans, VT) ENV-042 activity wheels with modular holding cages that are interfaced with a computer to record automatically the number of revolutions. The equipment was in a dedicated room that was separate from but nearby the housing room. The temperature, humidity, and lighting conditions were the same as those conditions in the open field activity room. The equipment consisted of eight activity wheels (35.6 cm diameter)

consisting of stainless steel grid rods (4.8 mm diameter) spaced 1.6 cm apart. Each activity wheel was connected to a separate plastic cage (48.26 cm L x 26.67 cm W x 20.32 cm D) with a stainless steel wire cover. Each cage had a 7.2 cm W x 10.2 cm H opening that allowed voluntary access to the running wheel or cage. Each activity wheel had 12 grams of drag. All cages had bedding in them to make them as similar as possible to the home cages. Animals were placed individually in the holding cage and were allowed access to the activity wheel for 2 hours. Revolutions of each activity wheel were recorded automatically on a dedicated computer that was interfaced with the activity wheels during a 2-hour access period. The data (number of quarter revolutions of the activity wheel) were electronically recorded in 120 1-minute bins. Holding cages and wheels were cleaned between subjects with 35% isopropyl alcohol solution. The running wheel activity testing was 120 minutes per day of testing.

Procedure

On the first day of the experiment, subjects were sequentially assigned to one of the four housing conditions: (1) isolated or non-enriched (NE); (2) physically-enriched (PE); (3) socially-enriched (SE); or (4) super-enriched (SUPER). There were 12 subjects in the NE condition, 12 in the PE condition, 12 in the SE condition, and 16 in the SUPER condition (the rat that died on day two was previously assigned to SUPER leaving 15 subjects in the SUPER condition). Based on Long (2008), it was determined that 12 subjects per condition was adequate. In order to replicate the super-enriched condition from Long (2008), 16 rats were used for the super-enriched condition in the present experiment.

Upon randomization, rats were assigned identification numbers and had their tails marked to show identification. Cages were numbered corresponding to the animals in that cage and rat tails were coded with a marker using a stripe system that corresponded to units of tens or ones, depending on the location of the mark on the tail. The part of the tail corresponding to the units of tens was the base of the tail, and the part of the tail corresponding to the units of ones was the end of the tails. Tail markings occurred two times a week. On each of the subsequent three days, each subject was briefly gentled (approximately 3 minutes each) to attenuate or prevent stress responses due to handling that was required to measure body weight and to place animals into the open field chambers and exercise apparatus.

Throughout the experiment, food consumption (FC) was measured every other day and body weight (BW) and Home Cage Activity 1 (HCA 1) were measured two times a week. Home Cage Activity 2 (HCA 2) was measured once a week. Open field (OF) was measured two times during the experiment (two weeks apart). Activity in exercise wheels (EX) was measured two times during the experiment (during the weeks when OF was not measured). All procedures occurred in the middle of the day (between 1300 hours and 1700 hours) throughout the experiment because the housing room was in its dark/active time during this time frame and also because this time frame was most convenient for investigators. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise. An equal number of animals from each housing condition were evaluated during each measurement. These differences are

reflected in the timeline below, such that OF (1/2) indicates that half of the animals' open field activities were measured on a given day, EX (1/4) indicate that a quarter of the animals' voluntary exercise were measured on a given day.

Experiment I Timeline	
Day	Measures taken
1	Rats arrive, BW, FC, MT, Assign housing, T&C, Gentling
2	Gentling
3	Gentling, FC
4	BW, HCA1, HCA2, MT, T&C
5	FC, Ex (acclimation)
6	Ex (acclimation)
7	BW, FC, HCA1, MT, T&C
8	OF (1/2)
9	FC, OF (1/2)
10	BW, HCA1, HCA2, MT, T&C
11	FC
12	--
13	FC
14	BW, HCA 1, MT, T&C, Ex (1/4)
15	FC, Ex (1/4)
16	Ex (1/4)
17	BW, FC, HCA1, HCA2, MT, T&C, Ex (1/4)
18	--
19	FC
20	--
21	BW, FC, HCA 1, MT, T&C
22	OF (1/2)
23	FC, OF (1/2)
24	BW, HCA1, HCA2, MT, T&C
25	FC
26	--
27	FC
28	BW, HCA 1, MT, T&C, Ex (1/4)
29	FC, Ex (1/4)
30	Ex (1/4)
31	BW, FC, HCA1, HCA2, MT, T&C, Ex (1/4)
32	--
33	FC
34	BW, FC, HCA 1, MT, T&C

BW = body weight; FC = food consumption; MT = mark tails; T&C = change toys and cages/tray; HCA = home cage activity; Ex = exercise wheel activity; OF = open field activity

Data Analytic Strategy for Experiment I

Subjects were assigned to housing conditions upon arrival. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent variable under study. Any significant main effects or interactions were examined using separate ANOVAs (Howell, 2007). If there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. In analyses where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. F values, degrees of freedom, and p values for analyses in Experiment I are provided in Appendix D.

Body weight and food consumption were analyzed using repeated-measures ANOVAs to assess changes over time throughout the experiment. Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field activity analyses, enrichment was the between-subjects factor and time was the within-subject factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers (i.e., horizontal activity, vertical activity, and center time). Although the three measures are conceptually related and could be analyzed with a multivariate ANOVA, the changes in these types of activity over time were of greater interest than how the three were related within a single open-field activity session. Within-session open-field activity also was analyzed using a repeated-measures ANOVA.

Home cage activity 1 was analyzed using ANOVAs for Experiment I. Three separate repeated-measures ANOVAs were computed for each of three different types of home cage activity (i.e., number of animals moving, amount of activity, and effort of activity). (Home cage activity 1 was not analyzed during Experiment IIa and IIb due to limitations of the measure. The home cage activity 1 measurement was based on the observation of each housing condition as a whole. The “cases” in the analyses of home cage activity 1 were housing conditions as a whole, rather than individual animals in each condition. Experiment IIa and IIb introduced another independent variable, drug condition. Because each housing condition contained animals with both nicotine and saline, observations based on housing conditions as a whole could be confounded because observations could be confounded by any drug effects.) Home cage activity 2 was analyzed using a repeated-measures ANOVA. Home cage activity 2 measured overall activity. This home cage activity measurement was based on activity of individual rats. Because the activity was measured for individual rats, this measure was not used on the super-enriched condition because it was too difficult to identify individual rats. Exercise was analyzed using a repeated-measures ANOVA. Enrichment was the between-subjects factor and time was the within-subjects factor.

In order to minimize the probability of Type I and Type II error, only if overall analyses were significant were subsequent analyses performed (Howell, 2007). All tests were two-tailed with significance determined by $p \leq 0.05$. In

addition, the experiment had adequate power (0.80), which minimizes Type II error (Howell, 2007).

Data were excluded from the analyses only if two criteria were met: (1) data points were more than three standard deviations from the mean of the experimental condition corresponding to those data, and (2) data were clearly inconsistent with the subject's other scores of the same variable over time. To determine inconsistency, each datum was compared with the subject's previous and next datum for that particular subject. If clearly disparate, the data were excluded from analyses. Twenty-four data points of 867 total data points (2.8%) were excluded from analyses from the food consumption data set, two data points of 561 total data points (0.3%) were excluded from analyses from the body weight data set, and four data points of out of 96 total data points (4.2%) were excluded from analyses from the home cage activity 1 data set; all of which met the above criteria. In addition, because of technological failures, eight data points of 102 total data points (7.8%) were not recorded for analyses from the locomotor data set.

Results for Experiment I

Body weight (see Figure 1). Animals began at approximately the same body weight. There was a significant main effect for time indicating that enriched and non-enriched animals gained weight over the course of the experiment ($F[1.391, 62.599] = 2861.98, p < 0.001$). There was a significant effect for housing, where the body weight of super enriched condition was significantly lower than the body weight of the non-enriched condition, while the physically enriched and

socially enriched conditions' body weight were in between and were not significantly different for the super and non-enriched conditions ($\text{Sup} \leq \text{PE} = \text{SE} \leq \text{NE}$) ($F [3, 45] = 3.32, p < 0.05$).

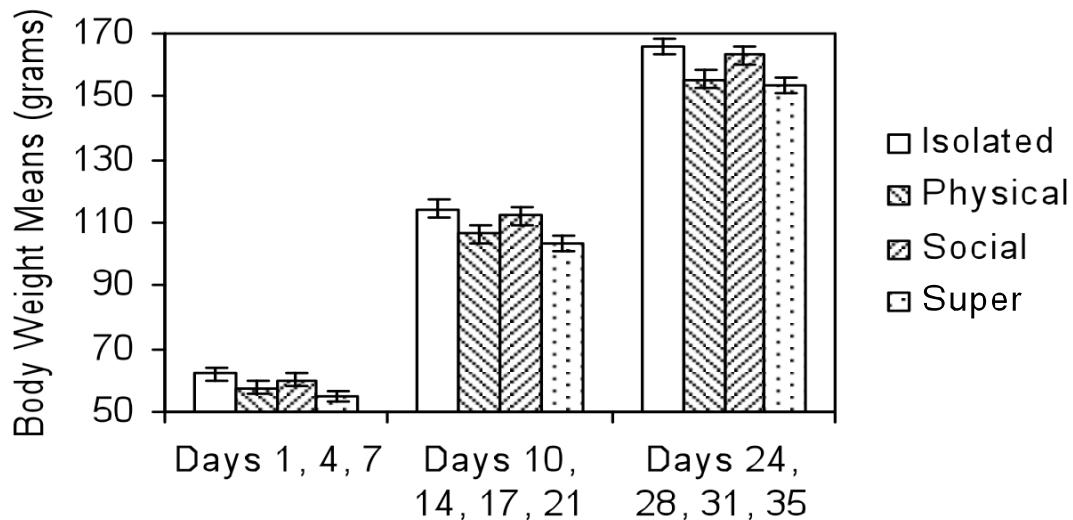


Figure 1. Mean body weights (SEM) of female Sprague Dawley rats in four different housing conditions

Food consumption (see Figure 2). At Day 3, an ANOVA revealed a significant difference in food consumption between housing conditions, where the super and socially enriched conditions had significantly lower food consumption than the physical and non-enriched conditions ($F [3, 47] = 6.008, p = 0.001$). Because of these housing differences at initial measurement, food consumption data analyses used the Day 3 values as covariates. There was a significant effect for time, such that all animals consumed more food over time ($F [5.527, 176.856] = 3.250, p < 0.001$). There was a significant effect for housing such that animals in the different housing conditions increased their rate of food consumption differentially. Specifically, the physically-enriched, socially-

enriched, and super-enriched animals all increased their food consumption at similar rates, but the non-enriched animals increased their food consumption at a greater rate ($PE=SE=Sup < NE$) ($F [3, 32] = 3.047, p < 0.05$). There was a significant time by housing interaction ($F [16.580, 176.856] = 2.625, p = 0.001$). Subsequent analyses revealed effects for housing during certain days, but not throughout the experiment. Significant effects for housing were detected for days 5, 7, 9, 11, 13, 15, 17, 19, 21, 27, and 33.

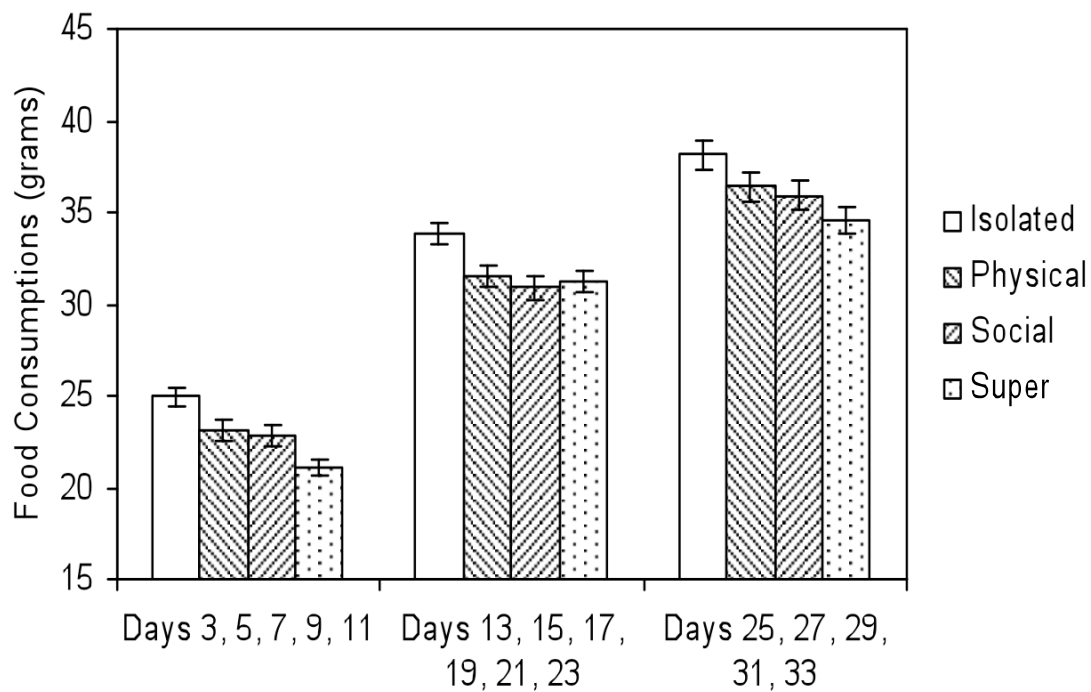


Figure 2. Mean food consumption ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

At Day 5, there was a significant housing effect, where the super-enriched and social-enriched ate significantly less than the non-enriched condition ($F [3, 40] = 10.077, p < 0.001$). At Day 7, there was a significant housing effect, where the super-enriched ate significantly less than the non-enriched condition but

there was no significant differences between the physically and socially-enriched conditions compared to other conditions ($F [3, 40] = 4.555, p < 0.01$). At Day 9, there was a significant housing effect, where the super-enriched and physically-enriched ate significantly less than the non-enriched condition ($F [3, 46] = 14.522, p < 0.001$). At Day 11, there was a significant housing effect, where the super-enriched, physically-enriched, and socially-enriched ate less than the non-enriched condition ($F [3, 46] = 7.68, p < 0.001$). At Day 13, there was a significant housing effect, where the super-enriched and physically-enriched conditions ate significantly less than the non-enriched condition ($F [3, 46] = 5.072, p < 0.01$). At Day 15, there was a significant housing effect, where the physically-enriched ate significantly less than the non-enriched condition ($F [3, 46] = 4.759, p < 0.01$). At Day 17, there was a significant housing effect, where the super-enriched condition ate significantly less than the non-enriched condition, and the physically and socially-enriched conditions did not differ significantly from other housing conditions ($F [3, 46] = 6.333, p = 0.001$). At Day 19, there was a significant housing effect, where the physically, socially, and super-enriched conditions ate significantly less than the non-enriched condition ($F [3, 46] = 4.165, p < 0.05$). At Day 21, there was a significant housing effect, where the physically, socially, and super-enriched conditions ate significantly less than the non-enriched condition ($F [3, 46] = 3.288, p < 0.05$). At Day 27, there was a significant housing effect, where the social-enriched and super-enriched ate significantly less than the non-enriched condition ($F [3, 39] = 7.225, p = 0.001$). At Day 33, there was a significant housing effect, where the social-

enriched and super-enriched ate significantly less than the non-enriched condition ($F [3, 46] = 4.687, p < 0.01$).

Open field activity (see Figures 3-7). Locomotor activity was measured in the open field chambers for 60 minutes, twice during each phase of the experiment. Horizontal activity provides an index of overall activity and health. Horizontal activity changes within a 60 minute session provide an index of simple learning and habituation. Vertical activity provides an index of exploration and/or escape. Changes in center time (relative to total time moving) provide an index of changes in anxiety with higher center time indicating lower anxiety.

For horizontal activity, there was a significant effect for housing, where the super-enriched condition had lower amounts of horizontal activity than the physically-enriched, socially-enriched, and non-enriched conditions ($\text{Sup} < \text{PE} = \text{SE} = \text{NE}$) ($F [3, 39] = 14.229, p < 0.001$). There was no effect for time and no time by housing interaction.

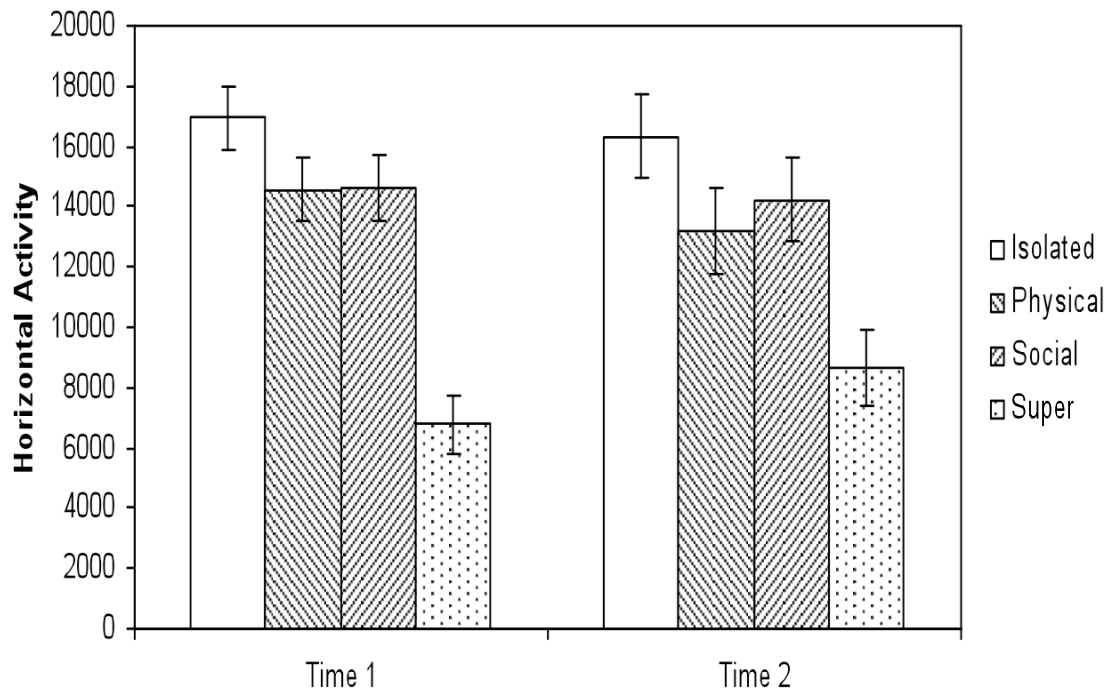


Figure 3. Mean open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

For vertical activity there was a significant effect time, where vertical activity increased over time for all conditions suggesting increased levels of exploration ($F [1, 39] = 12.594, p = 0.001$). There was a significant effect for housing, where the super-enriched condition had significantly lower vertical activity than the non-enriched and physically-enriched conditions, while the socially-enriched condition was not significantly different from any other housing conditions ($\text{Sup} < \text{PE} = \text{NE}$) ($F [3, 39] = 7.024, p = 0.001$). There was no time by housing interaction.

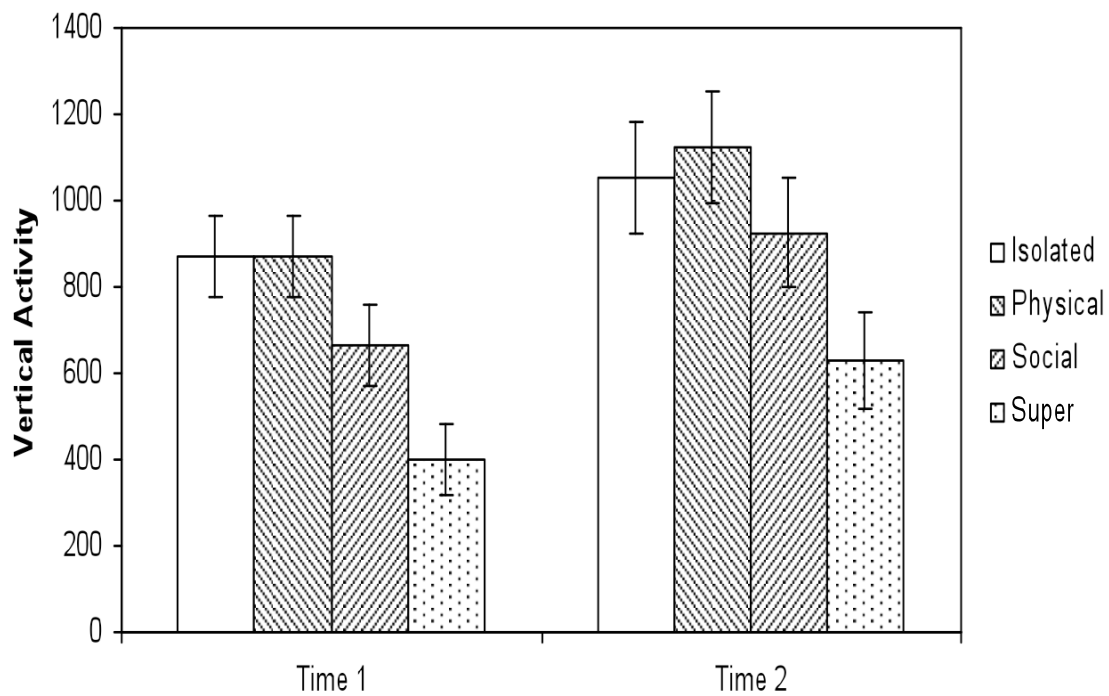


Figure 4. Mean open field vertical activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

For center time activity, there was a significant effect for housing, where the socially-enriched animals had greater center time activity than did super-enriched animals (SE>Sup) ($F [3, 39] = 4.051, p < 0.05$). There was no effect for time and no time by housing interaction.

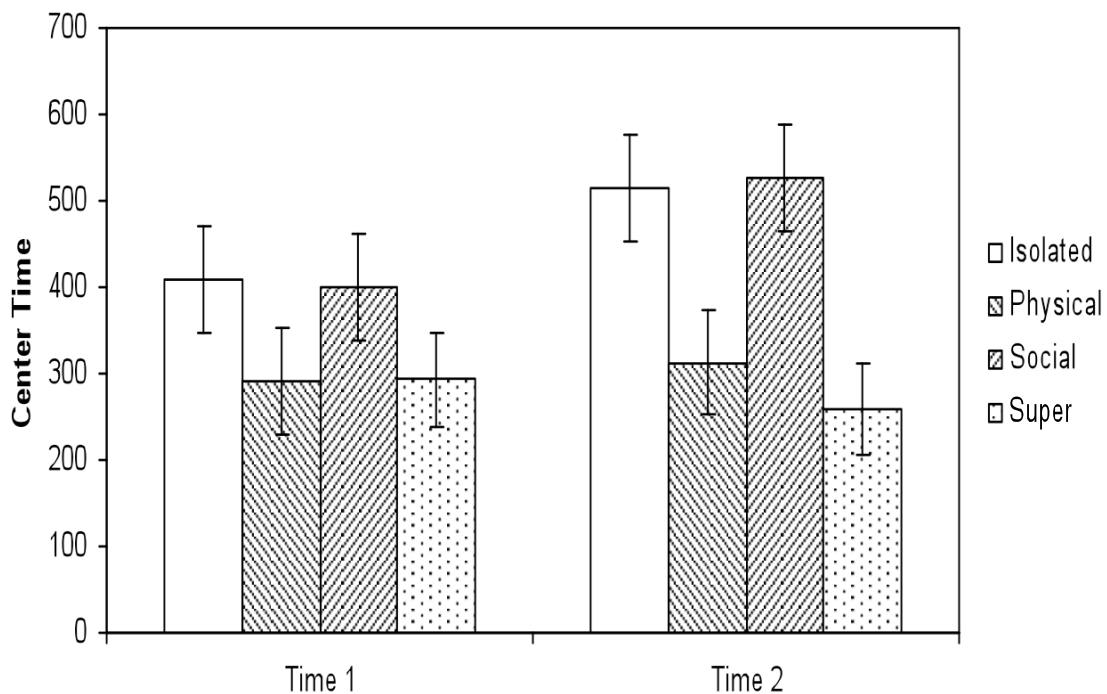


Figure 5. Mean open field center time ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

For the first within-session open field activity, there was a significant effect for time, where horizontal activity decreased over time ($F [7.055, 275.149] = 103.197, p < 0.001$). There was a significant effect for housing, where the super-enriched condition had lower amounts of activity than the physically-enriched, socially-enriched, and non-enriched conditions ($\text{Sup} < \text{PE} = \text{SE} = \text{NE}$) ($F [3, 39] = 20.583, p < 0.001$). There was a significant time by housing interaction, where horizontal activity decreased more quickly for the super-enriched condition compared to the other housing conditions over time ($F [21.165, 275.149] = 1.673, p < 0.05$).

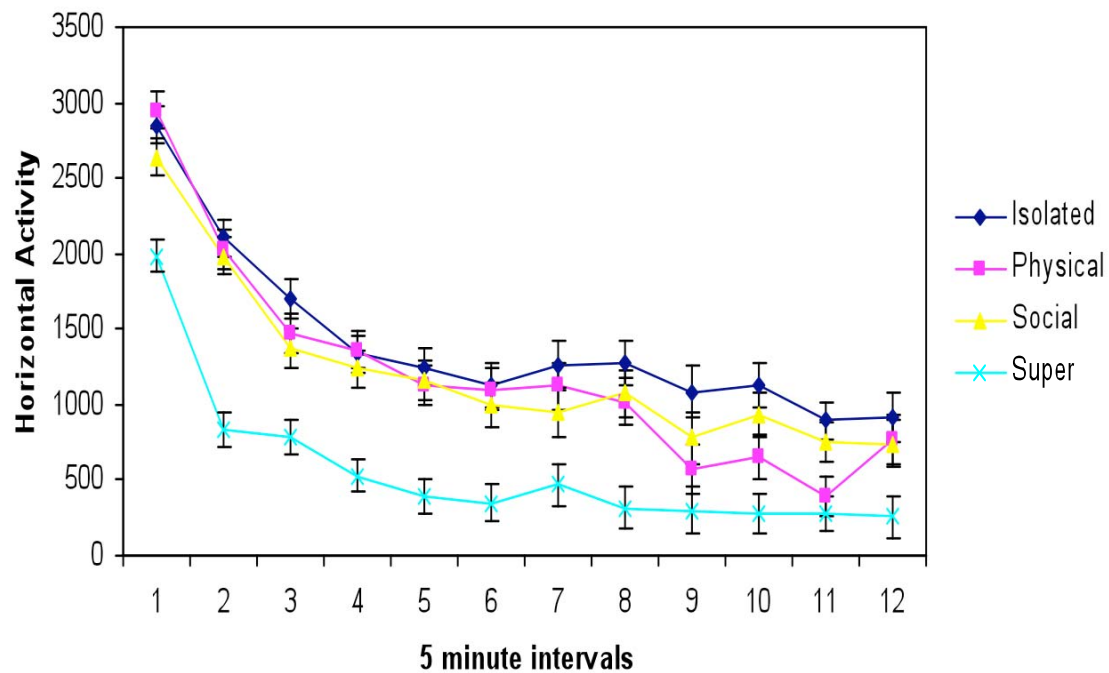


Figure 6. Mean within-session first open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

For the second within-session open field activity, there was a significant effect for time, where horizontal activity decreased over time ($F [6.787, 319.004] = 125.568, p < 0.001$). There was a significant effect for housing, where the super-enriched condition had lower amounts of activity than the socially-enriched and non-enriched conditions ($Sup < SE = NE$), but the physically-enriched condition did not differ from other housing conditions ($F [3, 47] = 7.396, p < 0.001$). There was a significant time by housing interaction, where the super-enriched condition decreased more quickly over time compared to the other housing conditions ($F [20.362, 319.004] = 2.148, p < 0.01$).

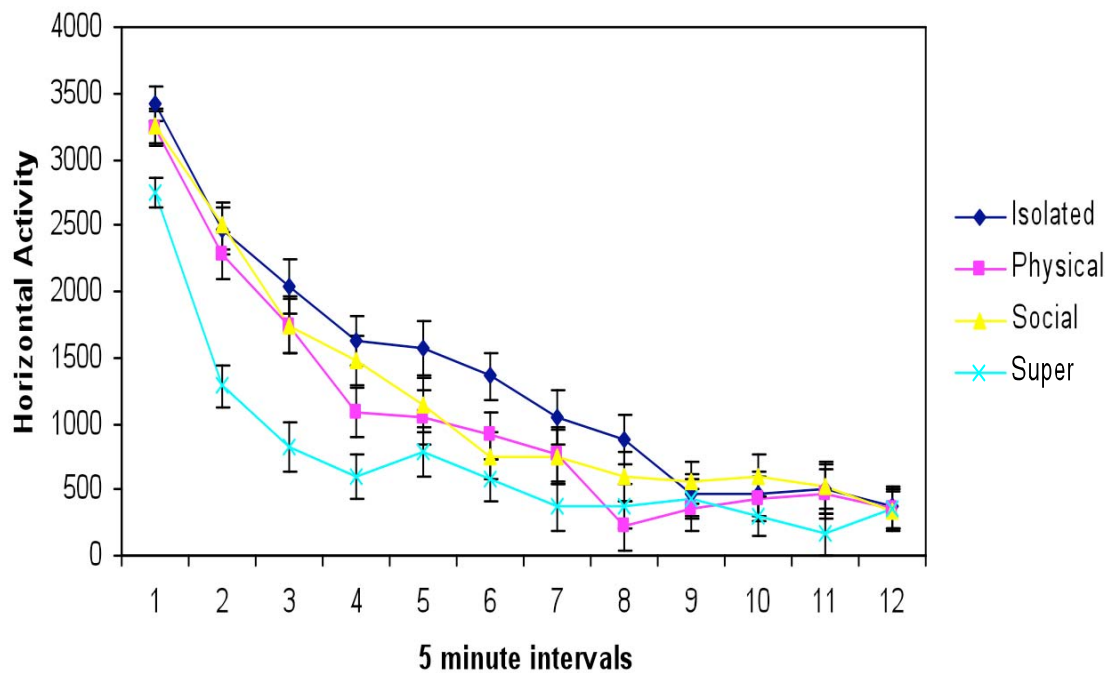


Figure 7. Mean within-session second open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

Home cage activity 1 (see Figure 8-10). Home cage activity was measured every other day by two independent raters each time. Home cage activity provides a unique opportunity to observe the animals in their home environment as opposed to other measures which entail observation in a novel environment (i.e., open field activity and exercise). Home cage activity has three subparts targeted to quantify three different aspects of activity: number of animals moving, amount of activity, and effort of activity.

Analyses for the number of animals moving revealed no significant time or housing effects and no significant time by housing interaction. For amount of activity, there was a significant effect for housing. The socially-enriched

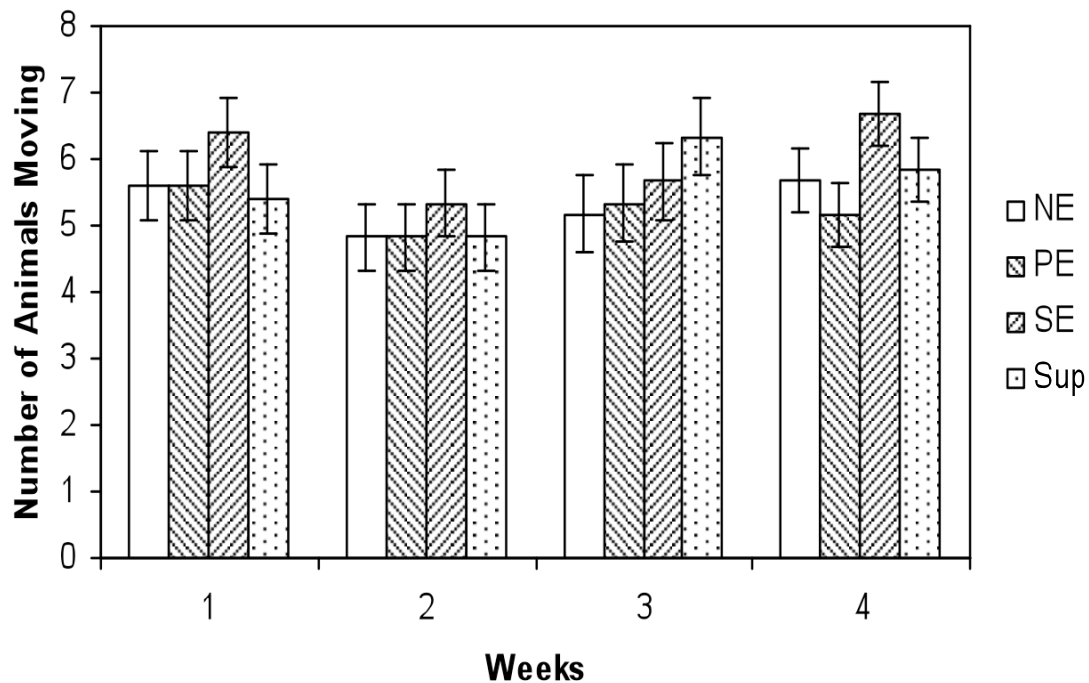


Figure 8. Mean number of animals moving within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions (NE = non enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

and super-enriched conditions had greater amounts of activity than did the non-enriched condition ($SE=Sup>NE$), and the super-enriched condition had greater amounts of activity than the physically-enriched condition ($Sup>PE$), but the physically-enriched condition did not differ from the socially-enriched condition ($PE=SE$) ($F [3, 16] = 10.237, p = 0.001$). There was no effect for time or time by housing interaction.

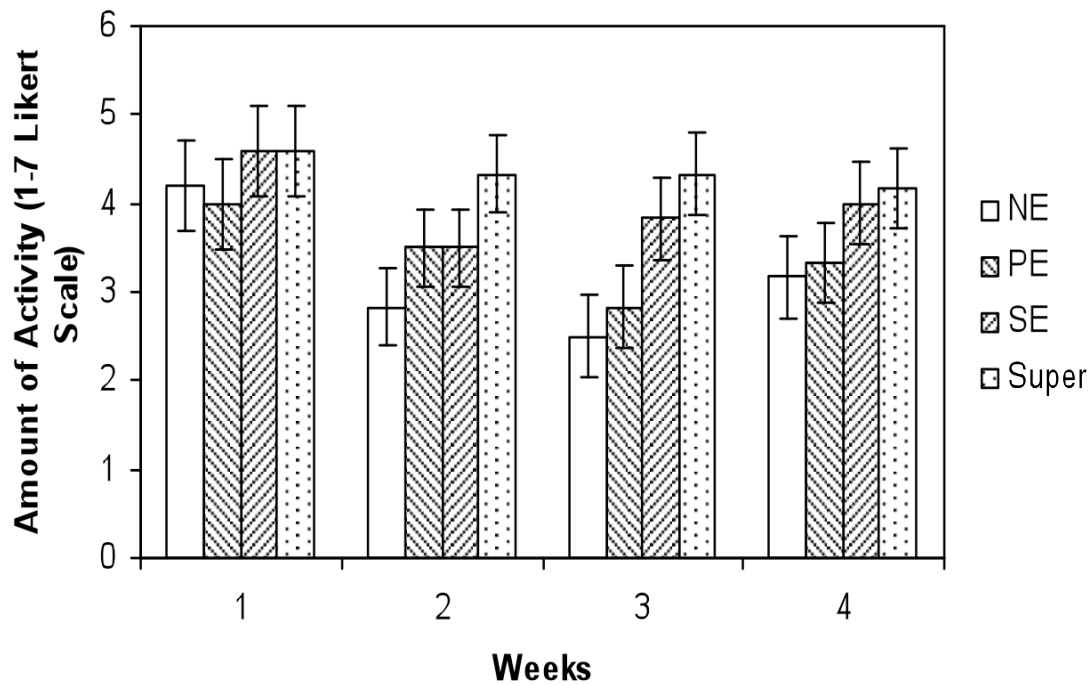


Figure 9. Mean amount of activity within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Super-enriched)

For effort of activity, there was a significant effect for housing, where the socially-enriched and super-enriched conditions had greater effort of activity than did the non-enriched and physically-enriched animals (Sup=SE>NE=PE) ($F [3, 16] = 13.235, p < 0.001$). There was no effect for time and no time by housing interaction.

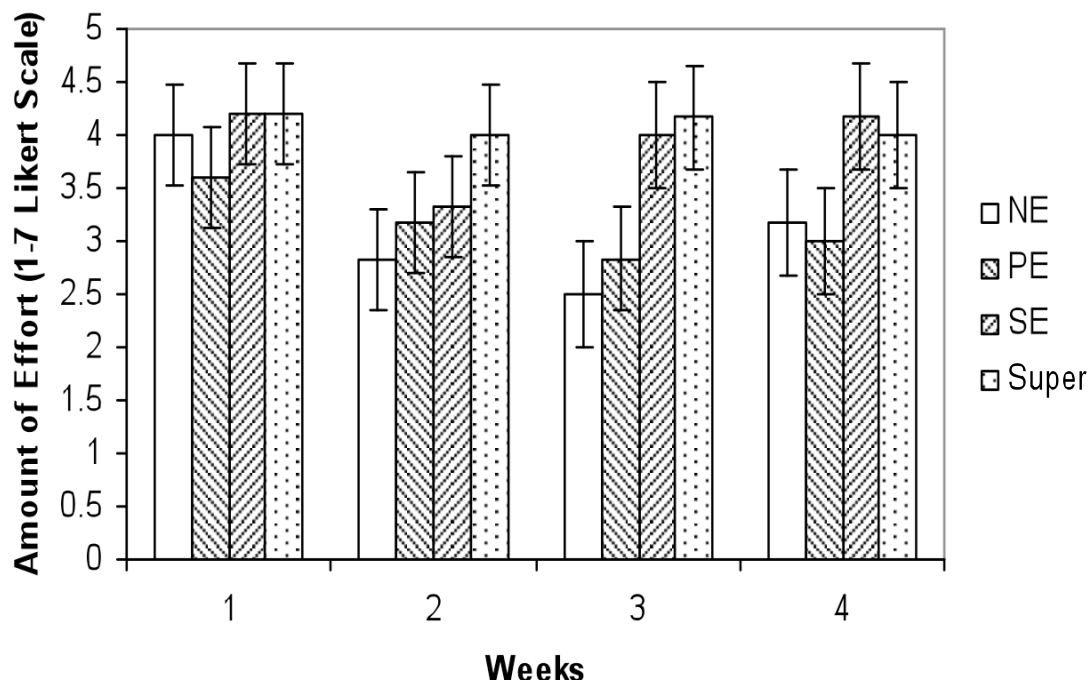


Figure 10. Mean amount of effort within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Super-enriched)

Home cage activity 2 (see Figure 11). Home cage activity was measured once a week by three independent raters each time. Home cage activity provides a unique opportunity to observe the animals in their home environment as opposed to other measures which entail observation in a novel environment (i.e., open field activity and exercise). Home cage activity 2 is different from home cage activity 1 in that observers recorded overall activity in each individual animal as opposed to trying to observe groups of animals in a complete housing condition at one time. It was not feasible to obtain data for home cage activity 2 on the super-enriched condition.

For overall activity, there was a significant effect for time, where activity changed in a similar pattern between housing conditions over time. Activity

increased from the first week to the second week, decreased from the second week throughout the fourth week, and increased again from the fourth week to the fifth week ($F [3.022, 99.727] = 15.982, p < 0.001$). There was no housing effect and no time by housing interaction.

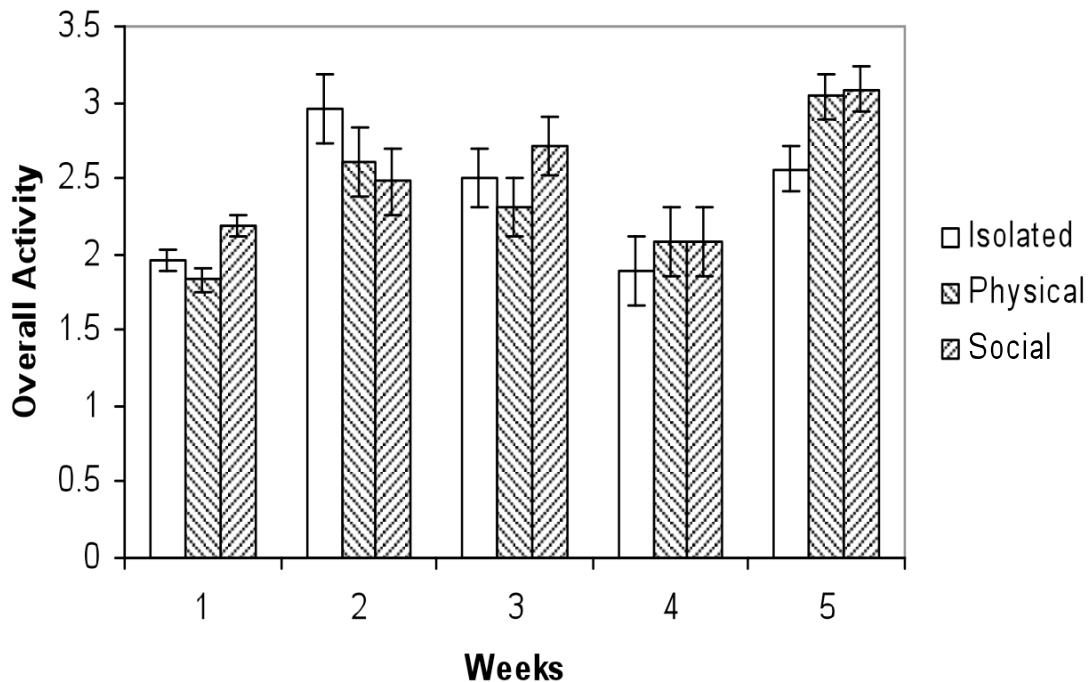


Figure 11. Mean overall activity within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in three different housing conditions

Exercise (see Figures 12). Exercise was measured in activity wheels attached to a plastic standard size cage for 120 minutes, twice during Experiment I. Exercise was considered voluntary as animals had free access to the activity wheels and could move freely between the activity wheel and the plastic cage. Bedding was added to the plastic cage to make it more comparable to the home cage. Total number of full revolutions were recorded electronically and indicated total amount of voluntary exercise.

Exercise increased over time among all housing conditions ($F [1, 46] = 12.298, p = 0.001$). There was no effect for housing or time by housing interaction.

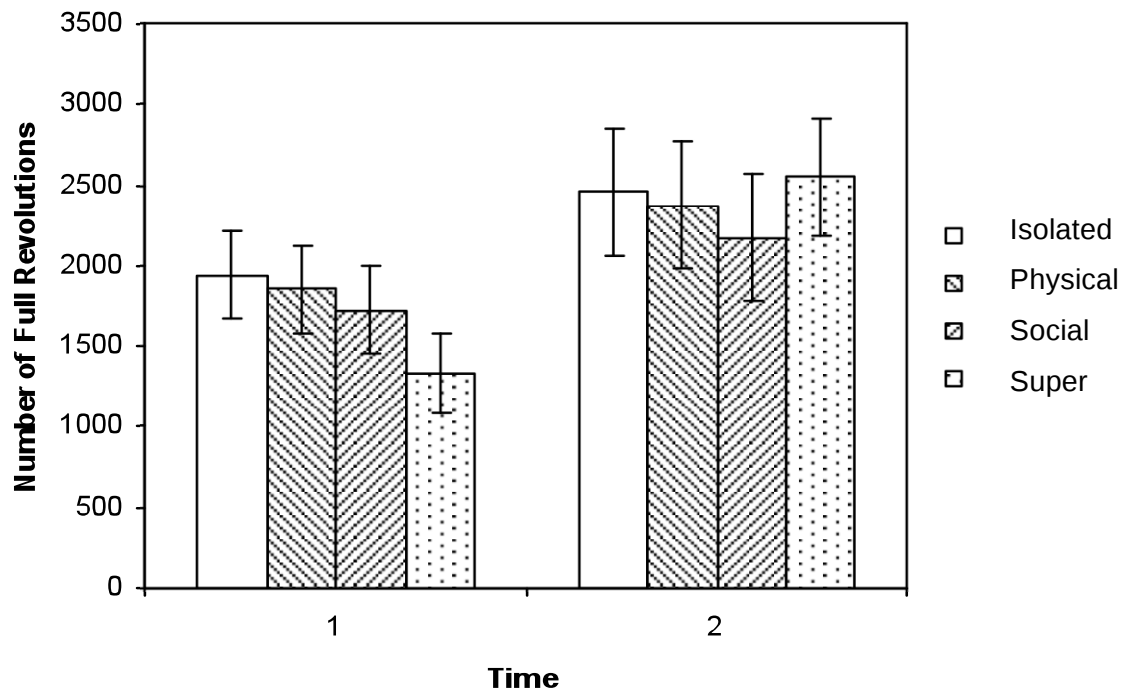


Figure 12. Mean voluntary exercise ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions

Discussion for Experiment I

The purpose of this experiment was to examine the effects of environmental enrichment on body weight, food consumption, and activity in female rats. Prior studies have reported that enrichment attenuates body weight gain, has minimal effects on food consumption, and has differing effects on activity depending on the activity measured.

Enrichment has been found to decrease open field activity and increase home cage activity for male and female rats (Tomchesson, 2006; Shafer 2006).

This effect on open field activity is hypothesized to reflect habituation in a novel environment while the increase in home cage activity has not yet been explained. Greater amounts of enrichment seem to have greater effects.

Long (2008) added a measure of voluntary exercise and reported similar effects of environmental enrichment on body weight, food consumption, and open field activity. However, socially-enriched rats initially decreased food consumption but then increased at the end of the experiment. Long (2008) attributed this surprising reversal to a longer duration of her experiment.

In addition, Long (2008) found that the environmental enrichment decreased home cage activity which was not consistent with previous findings. Further, environmental enrichment had differential effects on voluntary exercise depending on type and amount of enrichment. The physically-enriched condition increased voluntary exercise, the super-enriched decreased voluntary exercise, and the socially-enriched and non-enriched did not differ in voluntary exercise amounts.

The present experiment examined effects of housing conditions on body weight, food consumption, open field activity, home cage activity, and voluntary exercise in female rats. Environmental enrichment decreased food consumption, consistent with Tomchesson (2006) who studied male and female rats and Shafer (2006) who studied male rats. The reversal of food consumption that Long (2008) (who studied male rats) found in the socially-enriched rats was not found in the present study. This difference from Long (2008) may reflect true sex differences in environmental enrichment's effects over an extended period of

time (5 weeks) where male rats in the socially-enriched condition may increase food consumption but female rats continue to show environmental enrichment's effects of decreased food consumption.

The present experiment also found that environment enrichment, specifically conditions containing social enrichment, increased home cage activity consistent with Tomchesson (2006) and Shafer (2006), but different from Long (2008) who found that environmental enrichment decreased home cage activity. This finding may reflect a true sex difference in environmental enrichment on home cage activity.

There also were differences between the findings of the present experiment and previous work regarding environmental enrichment's effects on body weight, open field activity, and voluntary exercise. While Tomchesson (2006), Shafer (2006), and Long (2008) reported greater effects on open field activity and body weight with greater environmental enrichment, the present experiment found that only the super-enriched decreased open field activity and body weight gains. Unlike Long's (2008) findings on voluntary exercise, the present study did not find any differences in voluntary exercise among housing conditions (see Table 1). This difference in findings may suggest that physical enrichment does not beneficially affect exercise levels in females and super enrichment does not appear to be detrimental to exercise levels in female rats.

I. Environmental Enrichment		
	Males (Long, 2008)	Females
Body weight	↓	↓
Food consumption	↑	↓
Open Field Activity	↓	↓
Home cage activity	↓	↑
Exercise	↑	↔

Table 1. Comparison of the effects of environmental enrichment in male and female Sprague Dawley rats.

If the present findings generalize to humans, then there are some interesting implications relevant to women's health. Because super-enriched female rats had: decreased body weight gain, increased home cage activity, no decrease in food consumption, no increase in voluntary activity, and no increase in open field activity, perhaps it may be more important to influence women's body weight by increasing everyday activities rather than to focus on voluntary exercise.

Overall, these findings indicate that food consumption, body weight, and activity account for some body weight differences among enriched conditions. No enrichment had greater food consumption and therefore greater body weight, despite no differences in activity. This finding makes sense in that greater caloric intake without greater amounts of energy expenditure would increase body weight. In addition, combined physical and social enrichment decreased body weight by increasing common activities in females.

Experiment IIa

Overview

Experiment IIa examined the effects of nicotine on food consumption, body weight, and activity among female rats housed in four different housing conditions (NE, PE, SE, and SUPER). The influences of nicotine on energy intake and expenditure are well established. Nicotine attenuates weight gain, decreases food consumption, and slightly increases activity for male and female rats (Elliott et al., 2004; Faraday et al., 1999; Grunberg, Bowen, & Morse, 1984; Grunberg, 1982; Grunberg, 1985; Grunberg & Bowen, 1985; Grunberg, Winders, & Popp, 1987; Saah, Raygada, & Grunberg, 1994; Winders & Grunberg, 1989; 1990). Environmental enrichment's effects on body weight and food consumption are similar to the effects of nicotine in that enriched environments attenuate weight gain (Long, 2008; Shafer, 2006; & Tomchesson, 2006), but its effects on activity depend on the type of activity.

Recent research suggests that environmental enrichment attenuates the effects of acute or repeated acute nicotine on activity in male rats (Green et al., 2003; Elliott & Grunberg, 2005). However, Long (2008) found that chronic administration of nicotine in male rats attenuated body weight with an exaggeration in the PE condition, decreased food consumption except in the PE condition, increased activity in a curvilinear function such that $PE > SE > NE > SUPER$, and decreased activity in locomotor. Currently, it is unclear how environmental enrichment alters the effects of nicotine and if its effects are different depending on the way nicotine is administered (acute, repeated acute,

chronic). This experiment will determine if Long's findings on the effects of housing condition and chronic nicotine administration remain the same when using female rats.

Hypotheses

It was hypothesized that enriched housing would:

- (1) increase home cage activity, such that $PE > SE = SUPER = NE$. This hypothesis was based on the home cage activity findings of Tomchesson (2006) and the social enrichment findings of Elliott (2004) and Shafer (2006), who interpreted home cage activity as reflecting opportunities for movement in the presence of objects and others, and the findings from Brown and Grunberg (1995) that females had higher arousal when isolated than when in the presence of others. It was hypothesized that the physically-enriched rats would have the greatest increase in home cage activity. It was hypothesized that the socially enriched, super enriched, and non-enriched rats would have similar home cage activities because the socially enriched and super enriched rats could instigate activity in the other and the non-enriched rats could have a higher arousal (Brown & Grunberg, 1995) leading to more activity;
- (2) decrease open field activity, such that $SUPER < SE < PE < NE$. This hypothesis was based on the occurrence of habituation over time in the open field locomotor chamber within and between each session (Elliott & Grunberg, 2005; Elliott, 2004; Tomchesson, 2006; Shafer, 2006). It was hypothesized that the socially-enriched rats with have a greater decrease in open field activity than the physically-enriched rats;

(3) increase voluntary activity, such that $SUPER < SE < NE < PE$. This

hypothesis was based on the findings of Long (2006) on male rats;

(4) attenuate weight gain, such that $SUPER < SE < PE < NE$. This hypothesis was based on the findings of Tomchesson (2006), Shafer (2006), and Long (2008).

(5) have minimal effects on food consumption, such that $SUPER < SE < PE < NE$. This hypothesis was based on the food consumption findings of Tomchesson (2006) and Shafer (2006) and the idea that there would be no competition over food in the socially-enriched and super enriched environments because these environments were shown to be calming for females by Brown and Grunberg (1995).

It was hypothesized that nicotine would:

(1) decrease body weight. This hypothesis was based on many findings in our laboratory that nicotine decreases body weight for male and female rats (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989).

(2) decrease food consumption. This hypothesis was based on many findings in our laboratory that nicotine decreases food consumption for male and female rats (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989).

(3) increase open field activity. This hypothesis was based on many findings in our laboratory that nicotine increases open field activity in male rats (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg & Bowen, 1985).

(4) increase voluntary exercise. This hypothesis was based on many findings in our laboratory that nicotine increased open field activity (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg & Bowen, 1985). It was hypothesized that these effects on activity would generalize across all types of activity, including voluntary exercise.

Methods

Subjects

Subjects were the same 51 female Sprague Dawley rats (Charles River Laboratories) from Experiment I. The subjects were 56 days old at the beginning of this experiment and weighed between 143.4 and 222.4 grams with a mean of 179.09 grams.

General Husbandry

General husbandry remained the same as in Experiment I.

Independent Variables

Housing Conditions. Subjects were maintained in the same housing conditions they were assigned in Experiment I.

Drug Condition. Half the subjects from each housing condition received saline and half received nicotine.

Drug Administration

After Experiment I (which also served as a pre-drug phase for Experiment IIa), osmotic minipumps containing saline or 9 mg/kg/day of nicotine dihydrochloride were surgically implanted into subjects. The dosage of 9 mg/kg/day was used because it was within the effective dose-response curve

reported for effects of nicotine on body weight (Winders & Grunberg, 1989; Grunberg, 1992). Nicotine dihydrochloride was used based on previous reports (Grunberg & Bowen, 1985; Winders & Grunberg, 1990; Faraday, Scheufele, Rahman, & Grunberg, 1999; Scheufele, Faraday, & Grunberg, 2000; Elliott et al., 2004). Nicotine dihydrochloride was dissolved in physiologic saline (0.9% NaCl) and placed in Alzet osmotic minipumps (Model 2002, Durect Corporation). Surgeries were conducted in a separate Laboratory of Animal Medicine (LAM) procedure room equipped with anesthesia equipment and an operating table. Animals were anesthetized by inhalation anesthetic using LAM anesthetic equipment (vaporizer), isoflurane, oxygen, and a flowmeter. The percentage of isoflurane-oxygen mix was determined based on recommendations from (LAM) personnel and was approximately 4%. Subjects were placed inside an induction chamber saturated with isoflurane vapor. Subjects were removed from the chamber when they stopped moving and tail pinch produced no reflex movement (after approximately 2 minutes). Animals were placed on an absorbant surgical pad, and fitted with a nose cone attached to the vaporizer to deliver constant anesthesia during the entire surgical procedure. A 3 x 5 cm area between the withers (shoulder blades) was shaved with electric clippers and cleaned with the antiseptic Betadine. A 2 cm transverse incision within the shave region approximately 1 cm below the scapulae was made with blunt-nosed, curved-tipped Mayo surgical scissors, a pocket was created by gently spreading the subcutaneous tissues with the scissor tips, and the minipump was inserted with the pump opening toward the animal's posterior. Incisions were closed with two

to three 9 mm stainless steel wound clips. Subjects were observed until they were ambulatory. The order of the surgical procedures was counterbalanced to alternate nicotine and saline minipump implantation.

Dependent Variables

Activity Measurements: HCA, OF, Exercise. OF and Exercise measurements followed the same procedures as described under Experiment I. HCA 2 measurements were made once a week.

Body Weight and Food Consumption. BW and FC measurements followed the same procedures as described under Experiment I.

Procedures

Half of the subjects from each housing conditions received saline and half received nicotine. Animals from the NE and PE conditions were assigned to drug condition such that the body weights of the groups were comparable at the beginning of Experiment II. Animals from the SE and SUPER conditions were assigned to drug conditions by cage and an attempt was made to match body weights by cage. Drug was administered for 14 days via subcutaneously-implanted osmotic minipumps. Throughout the experiment, food consumption was measured every other day, and body weight was measured two times a week. HCA 2 was measured once a week. Open field was measured two times during the drug administration period (one week apart). Activity in exercise wheels was measured once during the drug administration period (during the week that OF was not measured).

Because of logistical considerations, not all animals' open field activity and voluntary exercise were measured on the same day. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise. An equal number of animals from each housing condition were evaluated during each measurement. These differences are reflected in the timeline below, such that OF (1/2) indicates that half of the animals' open field activity were measured on a given day, and EX (1/4) indicates that a quarter of the animals' voluntary exercise was measured on a given day.

Experiment IIa Timeline	
Day	Measures Taken
1	Implant (1/2)
2	FC, Implant (1/2)
3	OF (1/2), BW, HCA2, MT, T&C
4	FC, OF (1/2)
5	--
6	FC
7	BW, MT, T&C, Ex (1/4)
8	FC, Ex (1/4)
9	Ex (1/4)
10	FC, BW, HCA2, MT, T&C, Ex (1/4)
11	--
12	FC, OF (1/2)
13	OF (1/2)
14	FC, BW, MT, T&C

FC = food consumption; OF = open field activity; BW = body weight; HCA = home cage activity; MT = mark tails; T&C = change toys and cages/trays; Ex = exercise wheel activity

Data Analytic Strategy for Experiment IIa

Subjects maintained assignment to housing conditions from Experiment I. Subjects were assigned to drug condition as described above in the Procedures section. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent

variable under study. Any significant main effects or interactions were examined using separate ANOVAs (Howell, 2007). In analyses where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. If there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. F values, degrees of freedom, and p values for analyses in Experiment IIa are provided in Appendix E.

Body weight and food consumption were analyzed using repeated-measures ANCOVAs to assess over time throughout the experiment. The last body weight and food consumption measurements from the previous experiment were used as covariates.

Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field activity analyses, enrichment and drug condition were the between-subjects factors and time was the within-subject factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers (i.e., horizontal activity, vertical activity, and center time). Within-session open-field activity also was analyzed using a repeated measure ANOVA.

Home cage activity 2 was analyzed using a repeated-measures ANOVA. Home cage activity 2 measured overall activity. This home cage activity measurement was based on activity of individual rats. Because the activity was measured for individual rats, this measure was not used on the super-enriched condition because it was too difficult to identify individual rats.

Exercise was analyzed with a two-way ANOVA because only one exercise measurement was taken. Enrichment and drug condition were the between-subjects factors and time was the within-subject factor.

To minimize the probability of Type I and Type II error, only if overall analyses were significant were subsequent analyses performed (Howell, 2007). All tests were two-tailed with significance determined by $p \leq 0.05$. The experiment had adequate power (0.80), which minimized Type II error (Howell, 2007).

Data were excluded from the analyses only if two criteria were met: (1) data points were more than three standard deviations from the mean of the experimental condition corresponding to those data, and (2) data were inconsistent with the subject's scores over time. To determine inconsistency, each datum was compared with the subject's previous and subsequent datum for that particular subject. If clearly disparate, then the data were excluded from analyses. Thirty-two data points of 357 total data points (9%) were excluded from the food consumption data set, and 18 data points of 204 total data points (8.8%) were excluded from the body weight data set.

Results for Experiment IIa

Body weight (see Figure13). There was a significant effect for housing, where non-enriched animals had lower body weights than did animals from the socially-enriched and super-enriched housing groups, and physically-enriched animals had lower body weights than the super-enriched animals ($F [3, 27] = 3.296, p < 0.05$). There was a significant effect for drug condition, where animals

in the nicotine condition had lower body weights than did animals in the saline condition ($F [1, 27] = 18.581, p < 0.001$). A time by drug interaction was present ($F [1.824, 49.239] = 4.776, p < 0.05$), indicating that animals (specifically in the physically-enriched condition) in the nicotine condition exhibited attenuated weight gain as compared with animals in the saline condition.

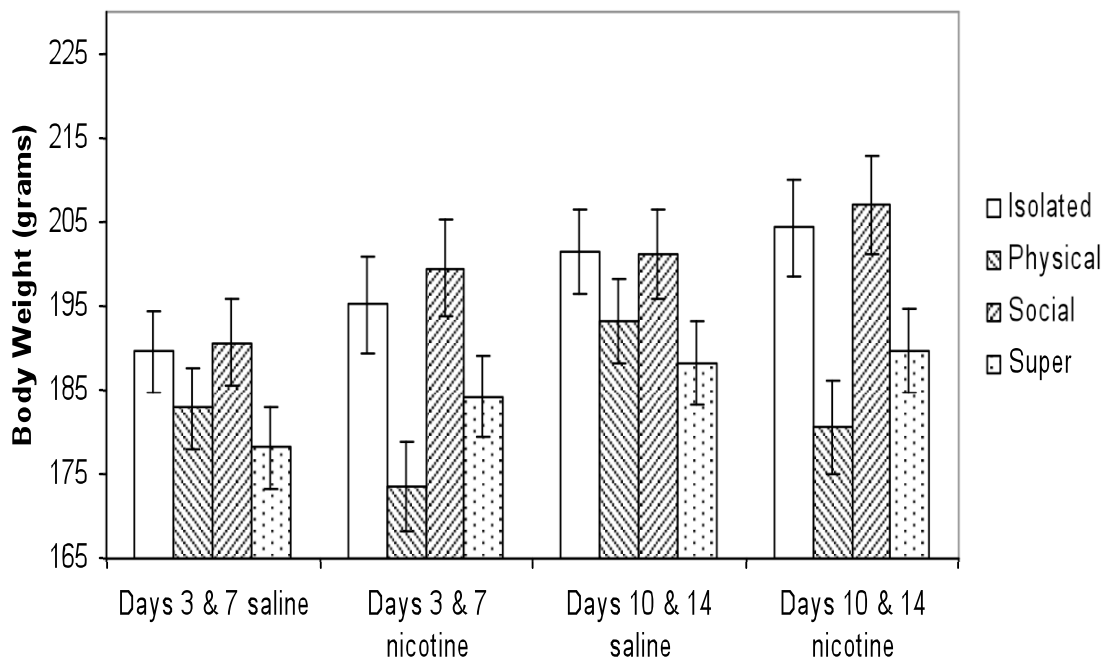


Figure 13. Mean body weight ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

Food consumption (see Figure 14). There was a significant effect for time ($F [2.592, 1703.382] = 8.668, p < 0.001$), but no effect for housing or drug.

Several interactions were evident: a time by housing interaction ($F [7.775, 1703.382] = 3.365, p = 0.001$), a time by drug interaction ($F [2.592, 1703.382] = 6.582, p = 0.001$), and a time by housing by drug interaction ($F [7.775, 1703.382] = 3.954, p = 0.001$).

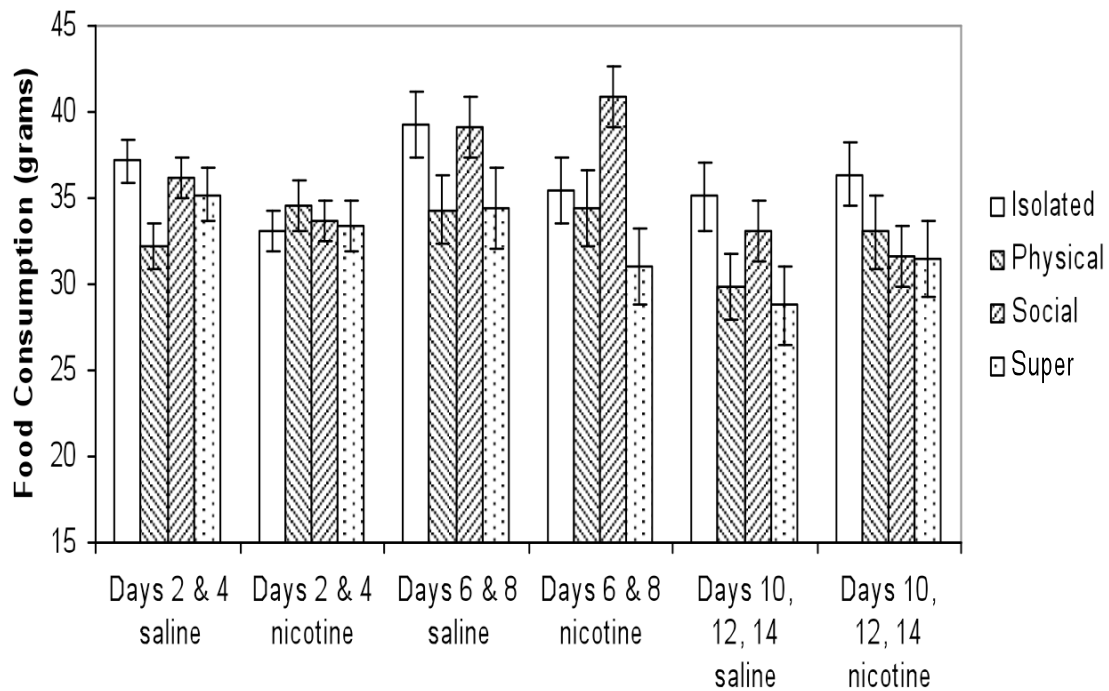


Figure 14. Mean food consumption ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

To understand these interactions, separate ANOVAs were conducted by day. At Day 4, there was a significant effect for drug, where overall animals in the saline condition consumed more food than the nicotine condition ($F [1, 42] = 14.504, p < 0.001$), and there was a significant drug by housing interaction, where the physically-enriched condition animals in the saline condition consumed less than animals in the nicotine condition ($F [3, 42] = 3.625, p < 0.05$). At Day 6 there were no significant differences. At Day 8, there was a significant effect for housing, where the non-enriched, physically-enriched, and socially-enriched conditions consumed more food than the super-enriched condition ($F [3, 37] = 7.696, p < 0.001$), and there was a significant drug by housing interaction, where the physically-enriched, socially-enriched, and super-enriched conditions animals

in the nicotine condition consumed more food, whereas animals in the saline condition ate more food in the non-enriched condition ($F [3, 37] = 3.749, p < 0.05$). At Day 10 there were no significant differences found. At Day 14, there was a significant effect for drug, where the animals in the nicotine conditions ate more food than the saline conditions ($F [1, 39] = 4.628, p < 0.05$), and there was a significant drug by housing interaction, where the saline animals in the socially-enriched condition ate more food than the nicotine animals ($F [3, 39] = 6.152, p < 0.01$).

In addition, the physically-enriched condition had a significant effect for time ($F [4, 24] = 4.798, p < 0.001$), where food consumption changed with no consistent pattern over time. In the socially-enriched condition there was a significant time by drug interaction ($F [4, 36] = 3.050, p < 0.05$), where food consumption was greater for saline animals on most days.

Open field activity (see Figures 15-19). For horizontal activity, there was a significant effect for housing, where the super-enriched animals engaged in lower amounts of horizontal activity than did the socially-enriched, physically-enriched, and non-enriched animals ($\text{Sup} < \text{SE} = \text{PE} = \text{NE}$) ($F [3, 43] = 19.609, p < 0.001$). There were no effects for time or drug. There also was a significant time by drug interaction, where animals in the saline condition increased amounts of horizontal activity over time while animals in the nicotine condition decreased amounts of horizontal activity over time ($\text{Sal} > \text{Nic}$) ($F [1, 43] = 5.743, p < 0.05$).

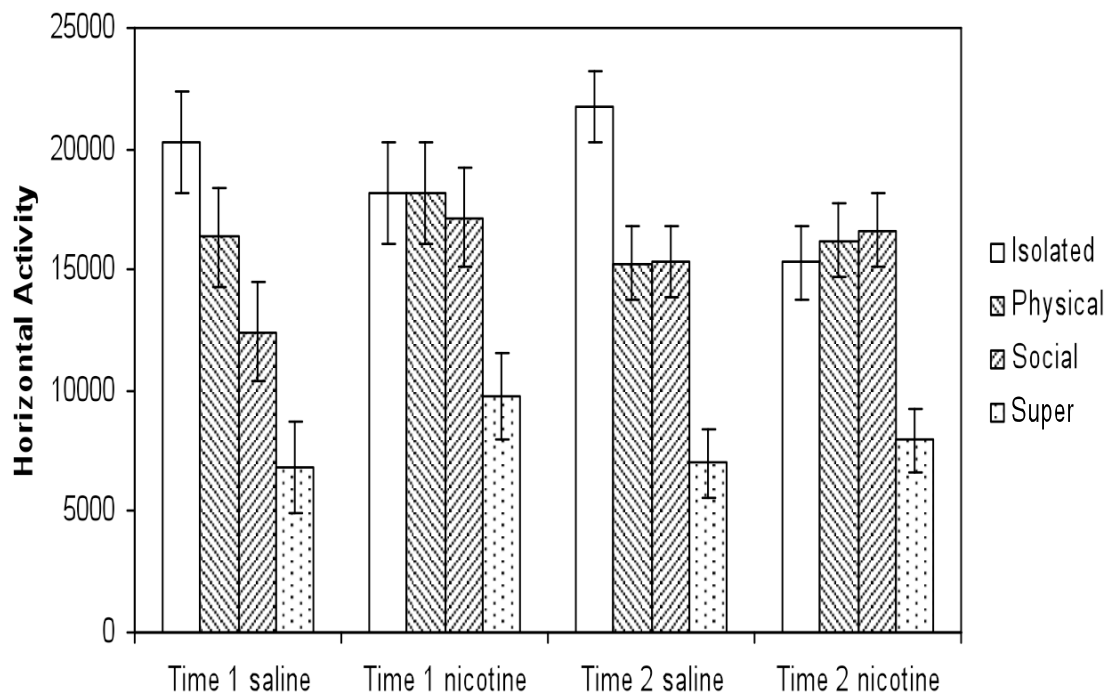


Figure 15. Mean open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For vertical activity, there was a significant effect for time, where vertical activity increased from the first open field measurement to the second open field measurement ($F [1, 43] = 8.096, p < 0.01$). There was a significant effect for housing, where animals in the super-enriched and socially-enriched conditions had lower amounts of vertical activity than the other housing conditions ($\text{Sup}=\text{SE}<\text{PE}=\text{NE}$) ($F [3, 43] = 12.231, p < 0.001$). There was no effect for drug. There was a significant time by drug interaction, where animals in the saline condition had greater amounts of vertical activity than did animals in the nicotine condition ($\text{Sal}>\text{Nic}$). Both saline and nicotine animals increased their amounts of vertical activity from the first open field measurement to the second open field measurement ($F [1, 43] = 5.643, p < 0.05$).

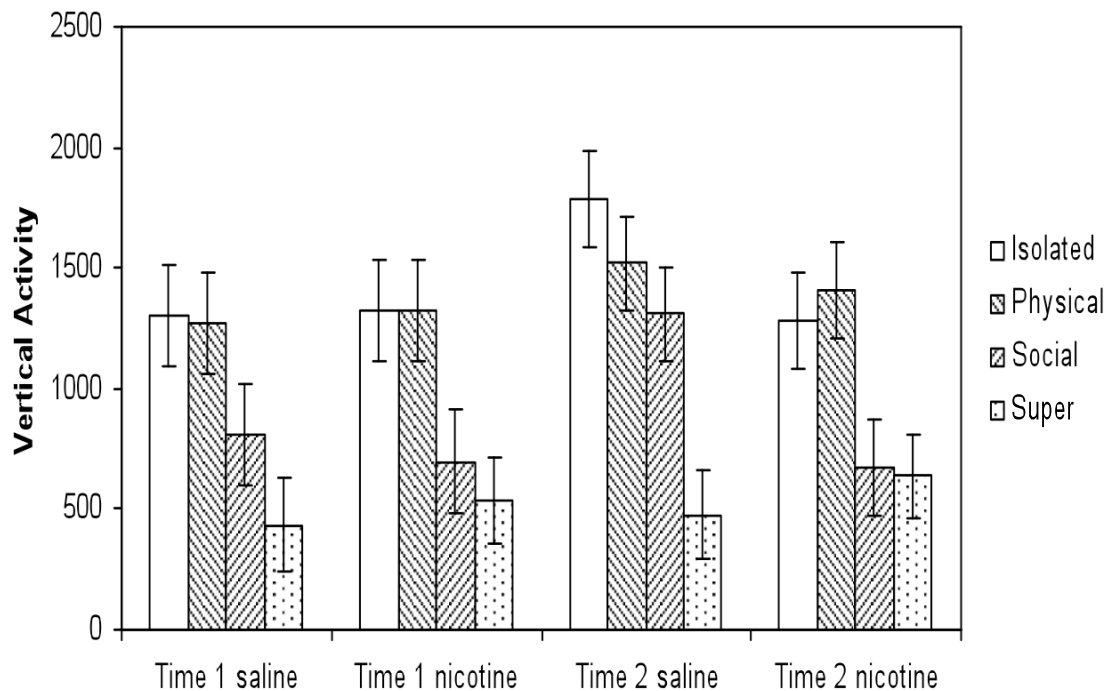


Figure 16. Mean open field vertical activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For center time, there was a significant effect for housing, where the super-enriched animals had less center time activity than the socially-enriched and non-enriched animals ($\text{Sup} < \text{SE} = \text{NE}$). Physically-enriched animals did not significantly differ from the other housing conditions ($F [3, 43] = 5.524, p < 0.01$). There was a significant time by drug interaction ($F [1, 43] = 5.057, p < 0.05$, a time by housing by drug interaction ($F [3, 43] = 3.667, p < 0.05$), where animals in the saline condition had increased center time activity over time, whereas animals in the nicotine condition had decreased center time activity over time, except in the physically-enriched condition where saline animals decreased center time activity over time and nicotine animals increased their center time activity over time.

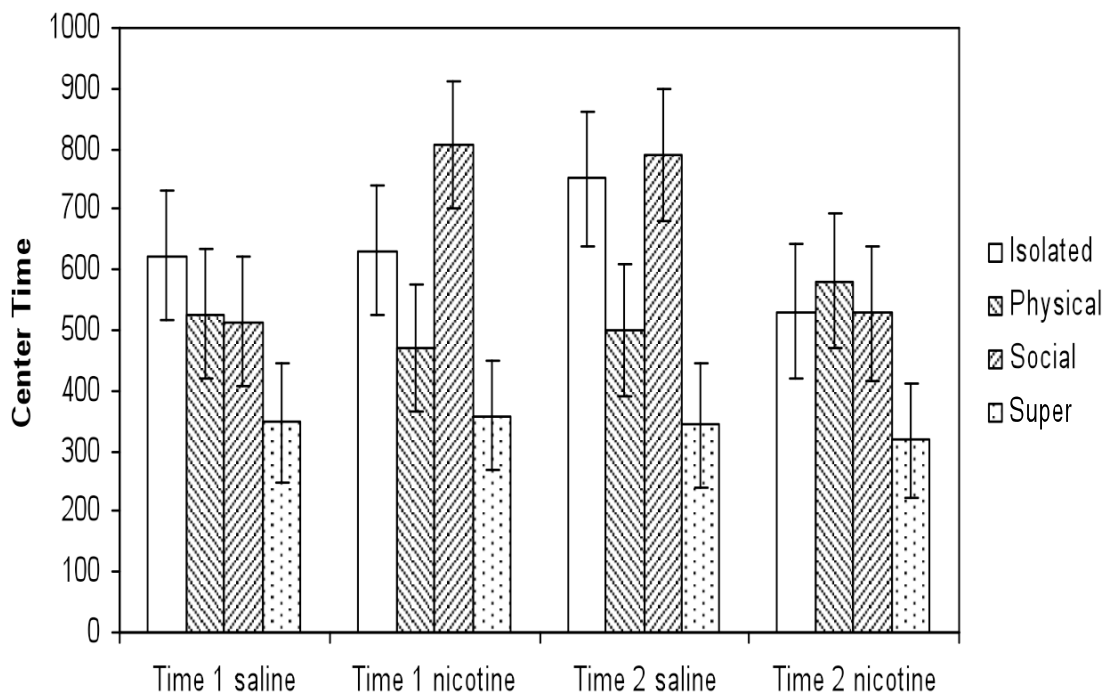


Figure 17. Mean open field center time activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For the first within-session open field activity (see Figure 18), there was a significant effect for time, where activity declined over time for all conditions ($F [6.314, 271.522] = 97.161, p < 0.001$), a significant effect for housing condition, where the non-enriched, physically-enriched, and socially-enriched conditions had greater amounts of activity than the super-enriched condition ($F [3, 43] = 12.087, p < 0.001$), but no effect for drug and no interactions.

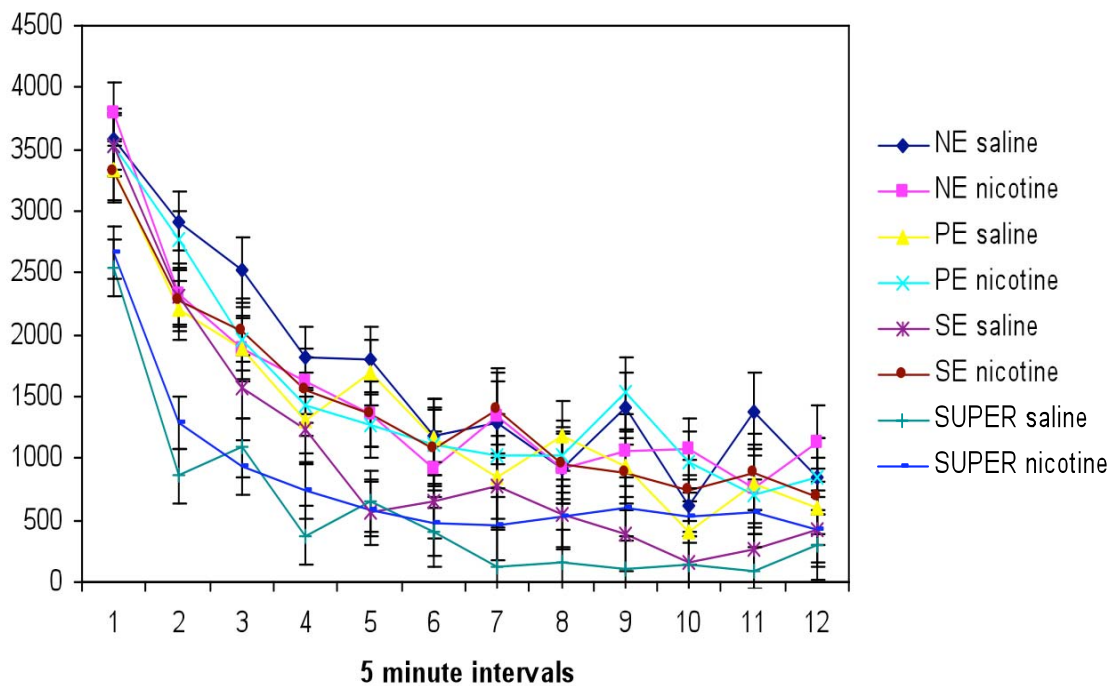


Figure 18. Mean within-session first open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two drug conditions

For the second within-session open field activity (see Figure 19), there was a significant effect for time, where all conditions decreased in activity over time ($F [6.757, 290.571] = 142.728, p < 0.001$), and a significant effect for housing condition, where the super-enriched condition had significantly lower amounts of activity than the physically-enriched, socially-enriched, and non-enriched conditions ($Sup < NE = SE = PE$) ($F [3, 43] = 23.229, p < 0.001$). There also was a significant time by housing interaction ($F [20.272, 290.571] = 2.673, p < 0.001$) and a housing by drug interaction ($F [3, 43] = 3.097, p < 0.05$). In the non-enriched condition, animals that were in the saline condition had greater amounts of activity than did the animals in the nicotine condition. In the

physically-enriched, socially-enriched, and super-enriched conditions, animals in the nicotine conditions had greater amounts of activity than did animals in the saline condition.

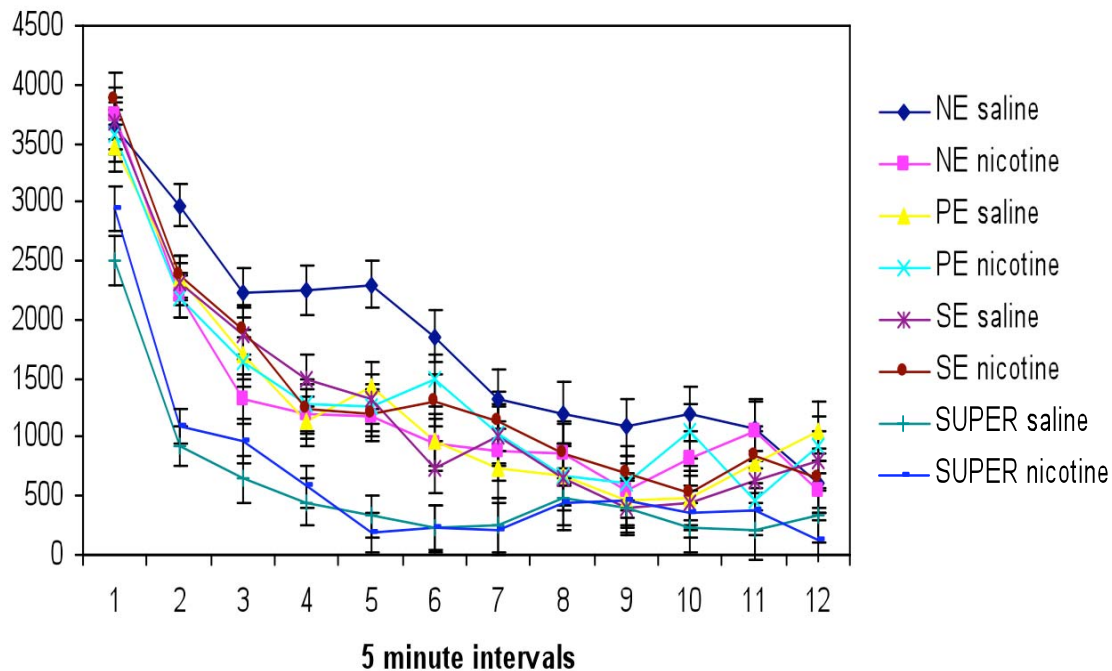


Figure 19. Mean within-session second open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two drug conditions

Home cage activity 2 (see Figure 20). It was not possible to obtain data for home cage activity 2 on the super-enriched condition or to differentiate between the saline and nicotine conditions within this housing condition.

For overall activity, there was a significant effect for time, where overall activity declined over time for all conditions measured ($F [1, 30] = 9.773$, $p < 0.01$). There was no housing or drug effect and no interactions.

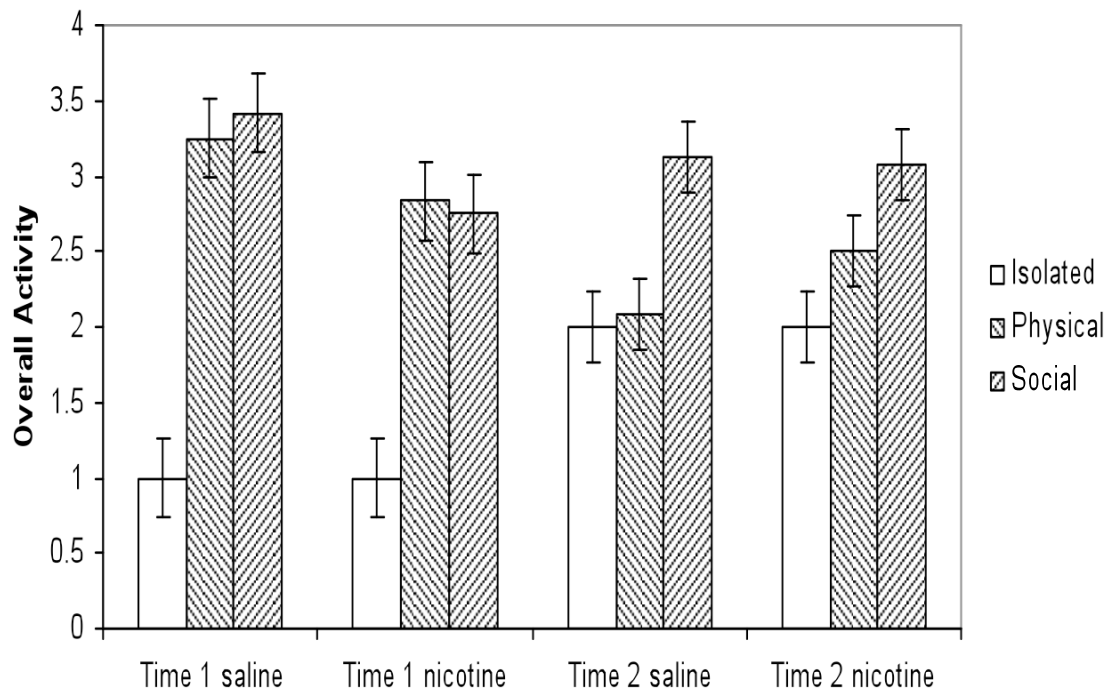


Figure 20. Mean overall activity within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in three different housing conditions and two different drug conditions

Exercise (see Figure 21). There was a significant housing by drug interaction, where the non-enriched, socially-enriched, and super-enriched conditions displayed greater amounts of exercise among the saline animals compared with the nicotine animals, but in the physically-enriched animals there were greater amounts of exercise by the nicotine animals than by the saline animals ($F [3, 43] = 3.634, p < 0.05$).

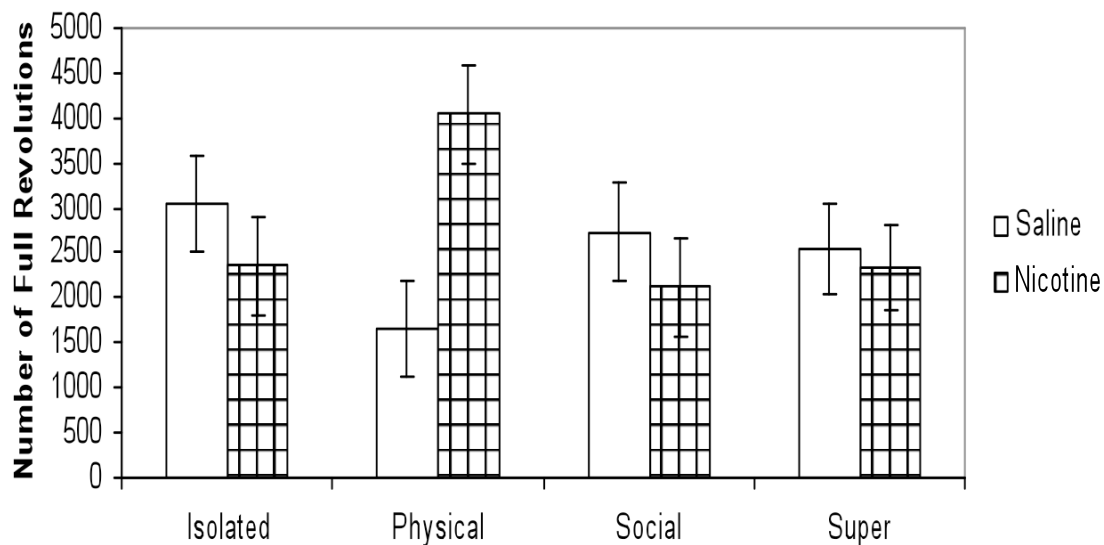


Figure 21. Mean voluntary exercise ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

Discussion for Experiment IIa

The purpose of this experiment was to examine the direct effects of environmental enrichment and nicotine on body weight, food consumption, and activity in female rats, as well as the effects of environmental enrichment on nicotine's effects on the same dependent variables. Previous experiments have examined environmental enrichment's effects on body weight, food consumption, and activity, as well as chronic nicotine's effects on the same dependent variables. However, Long (2008) was the first to examine both environmental enrichment and nicotine's effects together in male rats. This experiment is the first to examine these effects in female rats. Long (2008) found that environmental enrichment: attenuated weight gain, had no effect on food consumption, decreased open field activity, and the physically-enriched increased voluntary exercise while super-enrichment decreased voluntary

exercise. Long (2008) also found that nicotine: decreased food consumption, attenuated weight gain, and decreased vertical activity in open field. Long (2008) found that environmental enrichment potentiated nicotine's effects on food consumption in the physically and socially-enriched housing conditions.

The present experiment examined the dependent variables of food consumption, open field activity, home cage activity, and voluntary exercise to determine how environmental enrichment may alter nicotine's effects in female rats. Similar to past studies, the present experiment found that the super-enrichment decreased open field activity, and that nicotine attenuated weight gain compared to saline.

The present experiment had many differences from previous studies (see Table 2). The present experiment found that environmental enrichment did not attenuate gain; in fact the non-enriched had the lowest body weight gain. Environmental enrichment did not have differential effects on food consumption, and it did not have differences in home cage activity, although it is difficult to know what would have happened to the super-enriched if home cage activity could have been measured. The present experiment found that nicotine decreased horizontal activity as well as vertical activity in the locomotor chamber, and nicotine did not have effects on home cage activity. Interestingly, environmental enrichment increased horizontal activity in nicotine rats while no enrichment increased horizontal activity in saline rats. In combination with nicotine, the physically-enriched rats increased in voluntary exercise levels, whereas other housing conditions decreased in voluntary exercise levels

suggesting that nicotine is influential for increasing exercise in a physically-enriched environment.

Overall, nicotine's effects on body weight cannot be accounted for by food consumption or activity level differences. Environmental enrichment's effects on body weight cannot be fully accounted for by differences in food consumption and activity levels. The different findings, compared to Experiment I, on body weight as a result of environmental enrichment are difficult to explain, but they may be the result of developmental changes because the females during Experiment IIa have matured to young adulthood whereas they were adolescents in Experiment I. Further research needs to be conducted to examine this potential explanation. The lower body weights in the non-enriched and physically-enriched conditions are difficult to explain. Environmental enrichment and nicotine had several interactions, most interestingly the increase in voluntary exercise among nicotine rats in the physically-enriched environments. These findings may reflect true sex differences such that body weight in females may be more sensitive to the dosage of nicotine used, whereas some variables, such as food consumption and activity are not.

IIA. Nicotine		
	Males (Long, 2008)	Females
Body weight	↓	↓
Food consumption	↓	↔
Open Field Activity	↓	↓
Home cage activity	N/A	↔
Exercise	↑	↔

Table 2. Comparison of the effects of nicotine in male and female Sprague Dawley rats.

Experiment IIb

Overview

This experiment was designed to evaluate effects of nicotine cessation on physical activity, food consumption, and body weight of rats raised and living in different housing conditions. There were two different drug conditions (cessation of nicotine or saline). There were four different housing conditions that manipulated the social and physical environment. Physical activity was measured in three different ways: movement in an open field locomotor chamber, home cage activity, and activity in exercise wheels. This experiment included a two-week post-drug phase. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH, 1996).

Hypotheses

It was hypothesized that enriched housing would:

(1) decrease body weight, with super-enrichment having the greatest effects.

This hypothesis was based on the findings of Shafer (2006) and Tomchesson

(2006) who found that super-enrichment attenuates body weight gain.

(2) have minimal effects on food consumption. This hypothesis was based on the findings of Shafer (2006) and Tomchesson (2006) who found that enrichment had minimal effects on food consumption.

(3) decrease open field activity, with greater enrichment having greater effects (NE<PE=SE<Sup). This hypothesis was based on the findings of Shafer (2006)

and Tomchesson (2006) who found that enrichment, especially super-enrichment, decreased open field activity.

It was hypothesized that nicotine cessation would:

(1) increase body weight. This hypothesis was based on the findings of Grunberg, Bowen, and Winders (1986) and Perry (2007), who found that nicotine cessation resulted in increased body weight for male and female rats.

(2) increase food consumption. This hypothesis was based on the findings of Winders and Grunberg (1989) and Grunberg, Winders, and Popp (1987), who found that nicotine cessation resulted in increased food consumption for male and female rats.

(3) decrease open field activity. This hypothesis was based on the findings of Perry (2007), who found that nicotine cessation resulted in decreased open field activity for male and female Sprague Dawley rats.

(4) decrease voluntary exercise. This hypothesis was based on the findings of Perry (2007), who found that nicotine cessation resulted in decreased open field activity for Sprague Dawley rats. It was hypothesized that these findings would generalize to all forms of activity, including voluntary exercise.

Methods

Subjects

Subjects were the same 51 female Sprague Dawley rats (Charles River Laboratories) from Experiment I and IIa. The subjects were 71 days old at the beginning of this experiment and weighed between 158.5 and 253.4 grams with a mean of 204.4 grams.

General Husbandry

General husbandry remained the same as in Experiment IIa.

Independent Variables

Housing Conditions. Subjects were maintained in the same housing conditions that were assigned in Experiment IIa.

Drug Conditions. Subjects experienced cessation of nicotine or saline as assigned in Experiment IIa.

Dependent Variables

Activity Measurements: HCA, OF, Exercise. OF and Exercise measurements followed the same procedures as described under Experiment IIa. HCA 2 measurements were taken once a week.

Body Weight and Food Consumption. BW and FC measurements followed the same procedures as described under Experiment I.

Drug Cessation

After 14 days of saline or nicotine administration, the osmotic minipumps were surgically explanted and the post-drug phase began. Explant followed similar procedures as implant, except that the minipumps were removed.

Procedures

Animals were assigned to drug condition during Experiment IIa. These conditions were maintained through Experiment IIb. Osmotic minipumps were removed to begin the drug cessation phase. Throughout the experiment, food consumption (FC) was measured every other day. Body weight (BW) was measured two times a week. Open field (OF) was measured twice during the

drug cessation phase (one week apart). Activity in exercise wheels (EX) was measured once during the drug cessation phase. Because of logistical considerations, not all animals' open field activity and voluntary exercise were measured on the same day. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise, an equal number of animals from each housing condition were evaluated during each measurement. These differences are reflected in the timeline below, such that OF (1/2) indicate that half of the animals' open field activity were measured on a given day, and EX (1/4) indicate that a quarter of the animals' voluntary exercise were measured on a given day.

Experiment IIb Timeline	
Day	Measures Taken
1	Explant (1/2)
2	FC, Explant (1/2)
3	BW, HCA2, MT, T&C, OF (1/2)
4	FC, OF (1/2)
5	--
6	FC
7	BW, MT, T&C, Ex (1/4)
8	FC, Ex (1/4)
9	Ex (1/4)
10	FC, BW, HCA2, MT, T&C, Ex (1/4)
11	--
12	FC, OF (1/2)
13	OF (1/2)
14	--
15	Euthanasia

FC = food consumption; BW = body weight; MT = mark tails; T&C = change toys and cages/trays; OF = open field activity; Ex = exercise wheel activity

Euthanasia

All animals were sacrificed by Grunberg laboratory members by carbon dioxide inhalation following current LAM procedures. Subjects were placed in a standard rat cage (up to three at a time), and they were administered 100% carbon dioxide (Airgas Puritan Medical, Exp. 01-24-2012) at a maximum rate of 10-20% of chamber volume per minute. The carbon dioxide was released between 3.0-4.0 L per minute into the rat cage via a special lid. Heart samples were taken for further research.

Data Analytic Strategy for Experiment IIb

Subjects were maintained in housing and drug conditions from Experiment IIa. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent variable under study. Any significant main effects or interactions were examined using separate ANOVAs (Howell, 2007). In analyses where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. If there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. F values, degrees of freedom, and p values for analyses in Experiment IIb are provided in Appendix F.

Body weight and food consumption were analyzed using repeated-measures ANCOVAs to assess over time throughout the experiment. The last body weight and food consumption measurements from the previous experiment were used as covariates.

Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field

activity analyses, enrichment and drug condition were the between-subjects factors and time was the within-subject factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers. Within-session open-field activity also was analyzed using a repeated measure ANOVA.

Home cage activity 2 was analyzed using a repeated-measures ANOVA. Because the activity was measured for individual rats, this measure was not used on the super-enriched condition because it was too difficult to identify individual rats. Exercise was analyzed with a two-way ANOVA because only one exercise measurement was taken.

To minimize the probability of Type I and Type II error, only if overall analyses were significant were subsequent analyses be performed (Howell, 2007). All tests were two-tailed with significance determined by $p \leq 0.05$. The experiment had adequate power (0.80), which minimizes Type II error (Howell, 2007).

Data were excluded from the analyses based on the same criteria described for Experiment IIa. Nineteen data points of 306 total data points (6%) were excluded from the food consumption data set, and 15 data points of 153 total data points (9.8%) were excluded from the body weight data set.

Results for Experiment IIb

Body weight (see Figure 22). There was a significant effect for drug condition, where the animals in the nicotine cessation condition gained more than

did the animals in the saline cessation condition (Nic>Sal) ($F [1, 26] = 7.003$, $p < 0.05$). There was no effect for housing or time and no interactions.

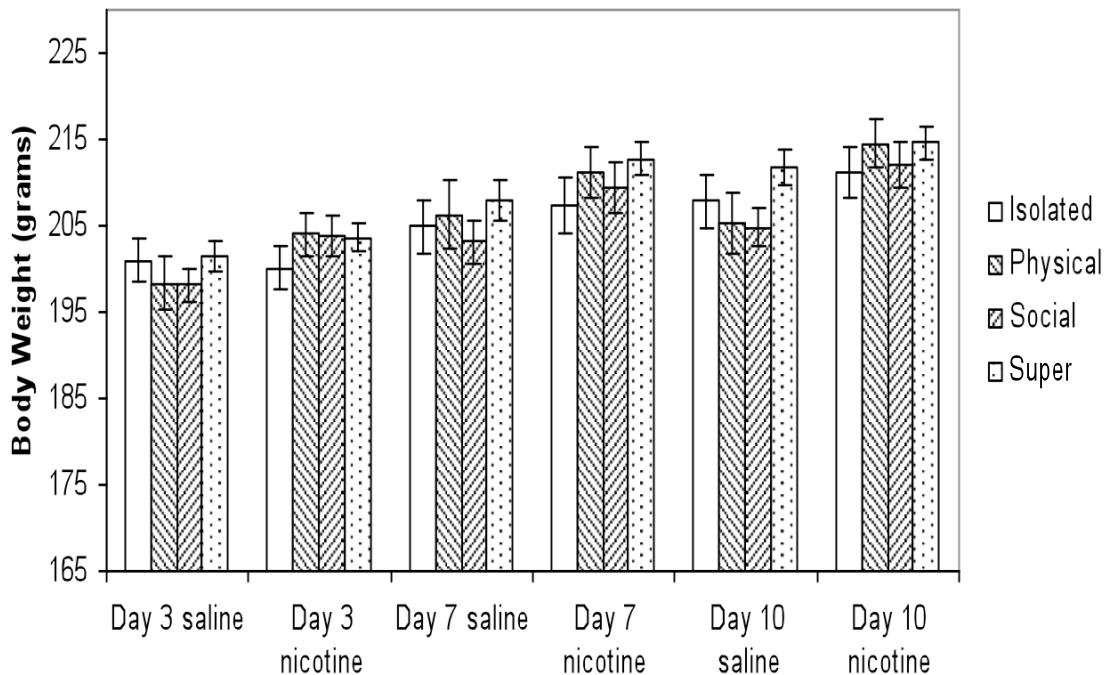


Figure 22. Mean body weight ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

Food consumption (see Figure 23). There was a significant effect for housing, where the super-enriched condition had lower amounts of food consumption than did the physically-enriched, socially-enriched, and non-enriched conditions, and overall the non-enriched condition consumed greater amounts of food than did the physically, socially, and super-enriched ($NE > PE \& SE > Sup$) ($F [3, 30] = 3.642$, $p < 0.05$). There was a significant effect for drug, where animals in the nicotine cessation condition ate more food than did the saline cessation animals (Nic>Sal) ($F [1, 30] = 30.607$, $p < 0.001$). There was a significant time by housing interaction ($F [7.326, 73.259] = 4.200$, $p = 0.001$).

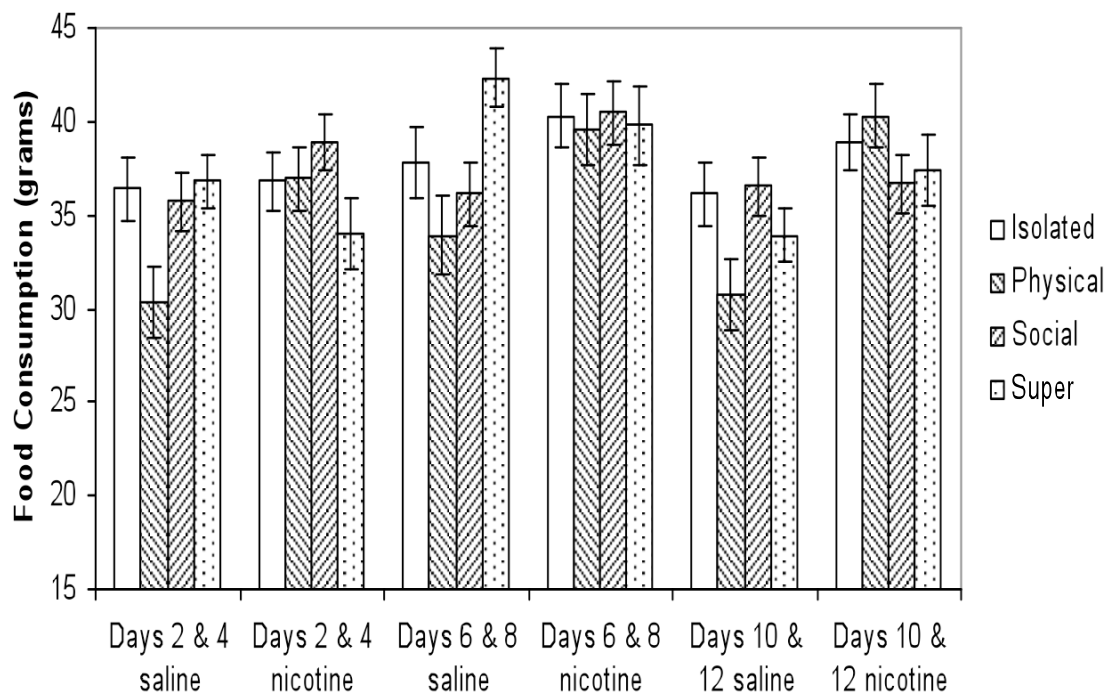


Figure 23. Mean food consumption ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

To understand the time by housing interaction, separate ANOVAs were conducted by day. There were no significant differences on Day 4 of Experiment IIb. At Day 6, there was a significant effect for housing, where animals in the non-enriched condition had greater amounts of food consumption than did the socially-enriched and super-enriched animals ($F [3, 39] = 3.228, p < 0.05$). At Day 8, there was a significant effect for housing, where the super-enriched condition had greater amounts of food consumption than did the non-enriched and socially-enriched conditions, but the physically-enriched condition consumed greater amounts of food than did the non-enriched condition ($F [3, 38] = 5.917, p < 0.01$). Day 10 did not reveal any significant differences. At Day 12, there was a significant housing effect, where the non-enriched condition consumed greater

amounts of food than did the physically and socially-enriched conditions ($F [3, 37] = 2.929, p < 0.05$).

Open field activity (see Figures 24-28). For horizontal activity, there was a significant effect for housing, where the non-enriched, physically-enriched, and socially-enriched animals all had greater amounts of horizontal activity than did the super-enriched animals ($\text{Sup} < \text{NE} = \text{PE} = \text{SE}$) ($F [3, 43] = 24.799, p < 0.001$). There was a significant effect for drug, where animals in the saline cessation condition had greater amounts of horizontal activity than did animals in the nicotine cessation condition ($\text{Sal} > \text{Nic}$) ($F [1, 43] = 9.647, p < 0.01$).

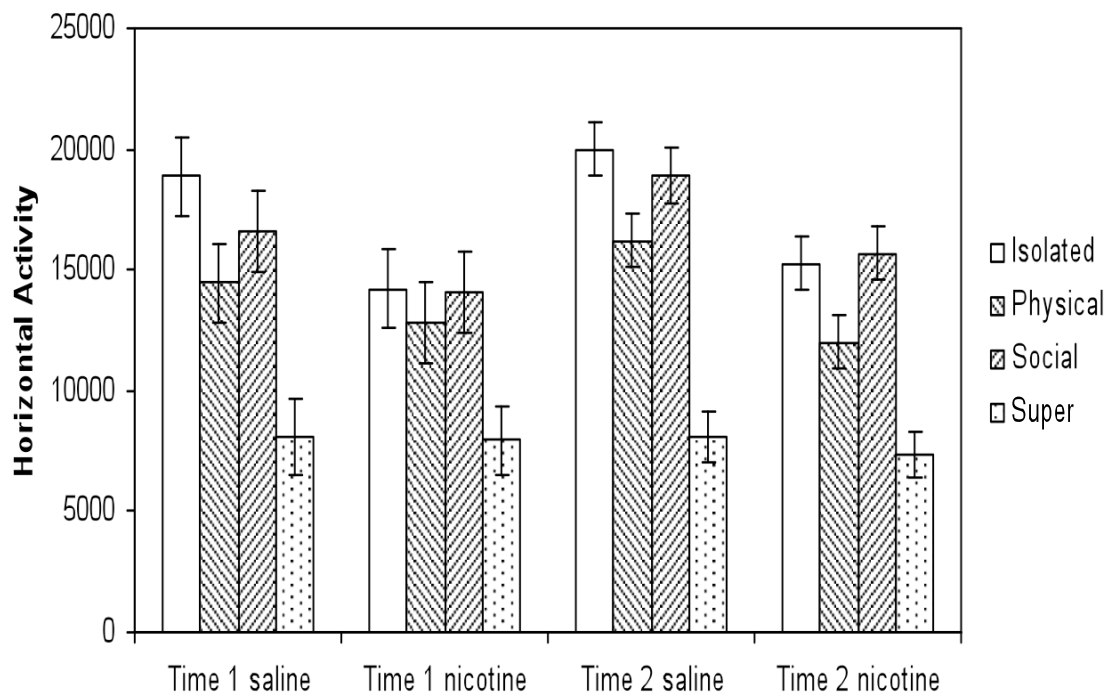


Figure 24. Mean open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For vertical activity (see Figure 25), there was a significant effect for housing, where the non-enriched, physically-enriched, and socially-enriched

animals all had greater amounts of vertical activity than did the super-enriched animals ($\text{Sup} < \text{NE} = \text{PE} = \text{SE}$) ($F [3, 43] = 21.352, p < 0.001$). There was a significant effect for time, where all housing conditions increased in amount of vertical activity from the first open field measurement to the second open field measurement ($F [1, 43] = 7.725, p < 0.01$). There was no effect for drug cessation condition and no interaction.

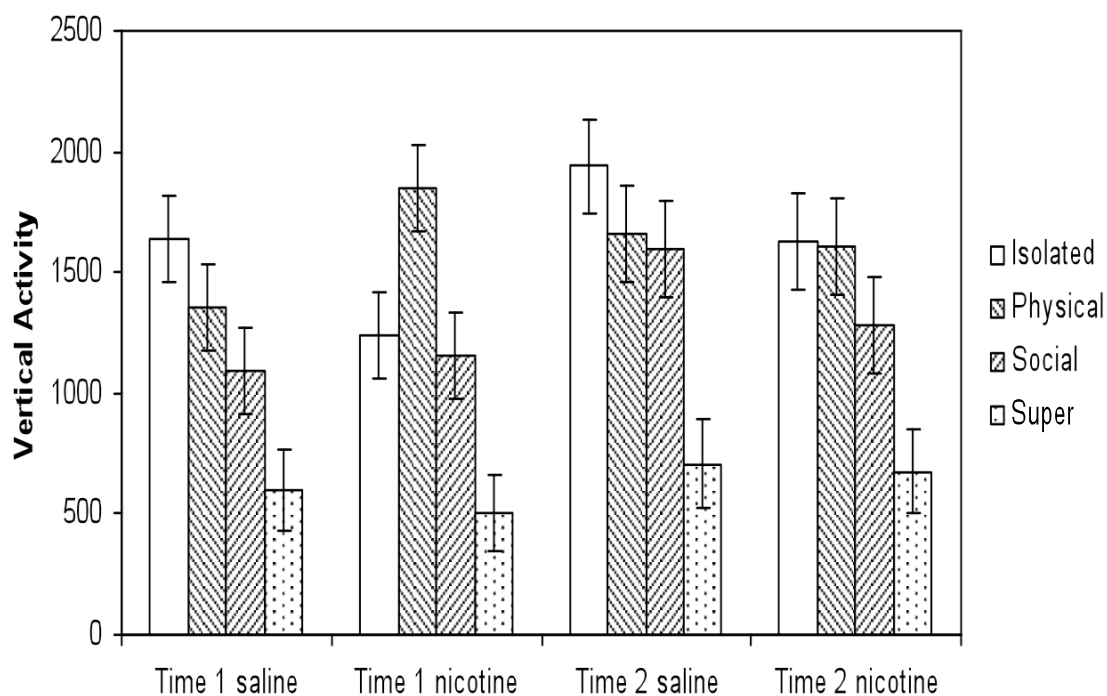


Figure 25. Mean open field vertical activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For center time activity (see Figure 26), there was a significant effect for housing, where the non-enriched condition had greater amounts of center time than did the super-enriched condition ($F [3, 43] = 3.884, p < 0.05$). There was a significant effect for drug, where saline cessation animals had greater amounts of

center time than did nicotine cessation animals (Sal>Nic) ($F [1, 43] = 9.074, p < 0.01$).

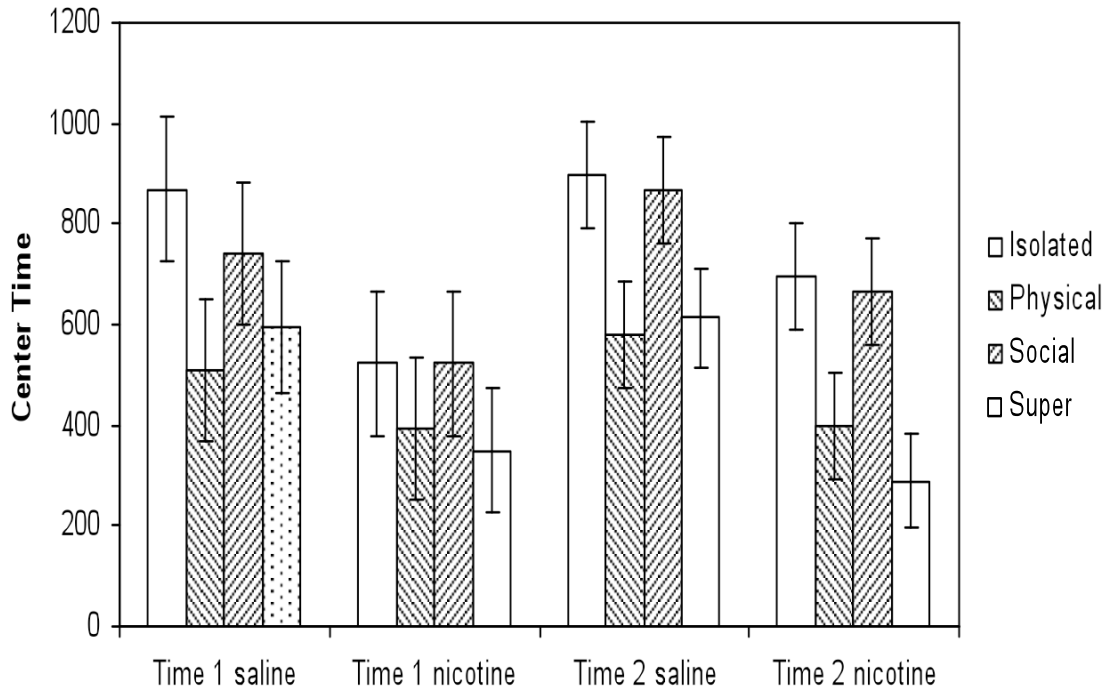


Figure 26. Mean open field center time ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

For the first within-session open field activity (see Figure 27), there was a significant effect for time, where all housing conditions activity declined over time ($F [7.039, 302.670] = 153.378, p < 0.001$). There was a significant effect for housing condition, where the non-enriched, physically-enriched, and socially-enriched conditions all had greater amounts of activity than the super-enriched condition (Sup<NE=SE=PE) ($F [3, 43] = 12.757, p < 0.001$). There was no effect for drug. There was a significant time by housing interaction ($F [21.117, 302.670] = 3.634, p < 0.001$).

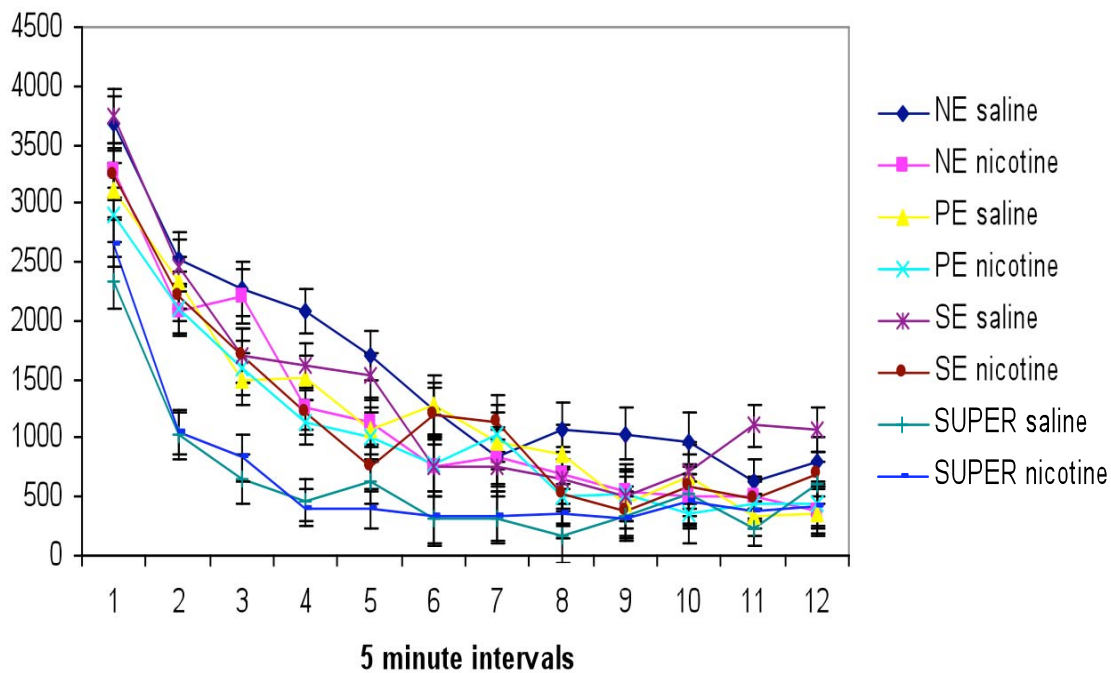


Figure 27. Mean within-session first open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two drug conditions

For the second within-session open field activity (see Figure 28), there was a significant effect for time, where activity decreased over time in all conditions ($F [7.229, 310.844] = 134.615, p < 0.001$). There was a significant effect for housing condition, where the non-enriched, physically-enriched, and socially-enriched conditions all had greater amounts of activity than the super-enriched condition ($\text{Sup} < \text{NE} = \text{PE} = \text{SE}$) ($F [3, 43] = 38.635, p < 0.001$). There was a significant effect for drug, where the saline cessation condition also had greater amounts of activity than did the nicotine cessation condition ($\text{Sal} > \text{Nic}$) ($F [1, 43] = 17.329, p < 0.001$). There also was a significant time by housing interaction ($F [21.687, 310.844] = 1.671, p < 0.05$).

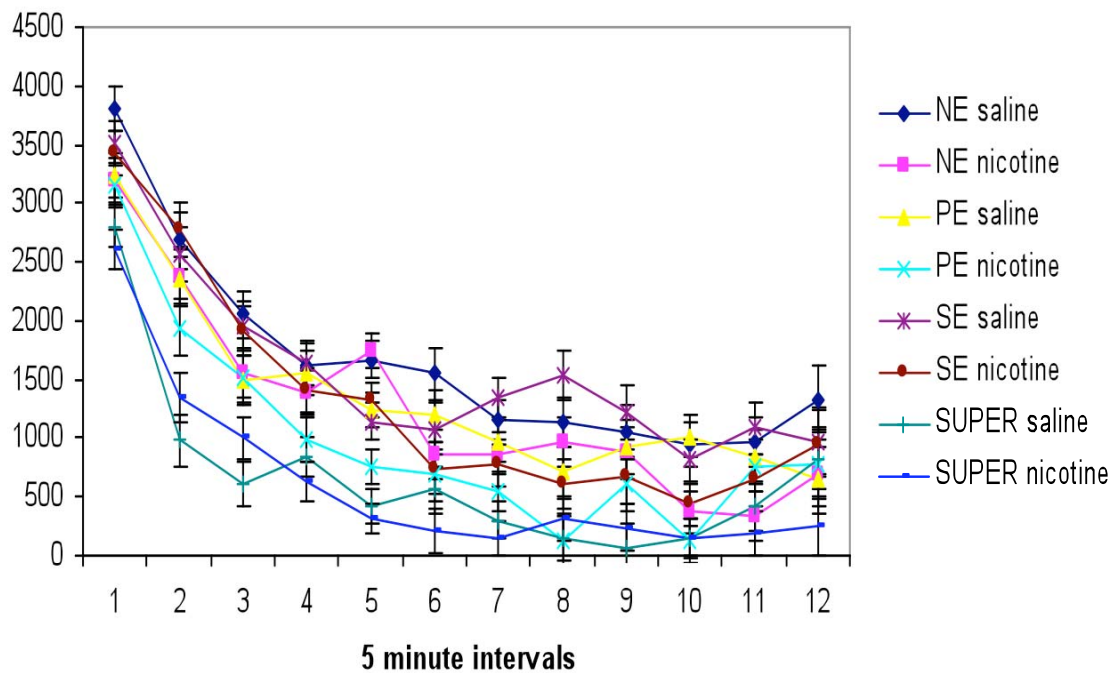


Figure 28. Mean within-session second open field horizontal activity ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two drug conditions

Home cage activity 2 (see Figure 29). It was not possible to obtain data for home cage activity 2 on the super-enriched condition or to differentiate between the saline and nicotine cessation conditions in this housing condition.

For overall activity, there was a significant effect for time, where overall home cage activity increased over time ($F [1, 30] = 8.356, p < .01$). There was a significant effect for housing, where the socially-enriched condition had greater amounts of activity than did the physically-enriched and non-enriched conditions ($SE > PE = NE$) ($F [2, 30] = 14.124, p < 0.001$). There was a significant time by housing interaction, where in the non-enriched condition only, activity decreased

from the first measurement to the second measurement ($F [2, 30] = 5.941, p < 0.01$).

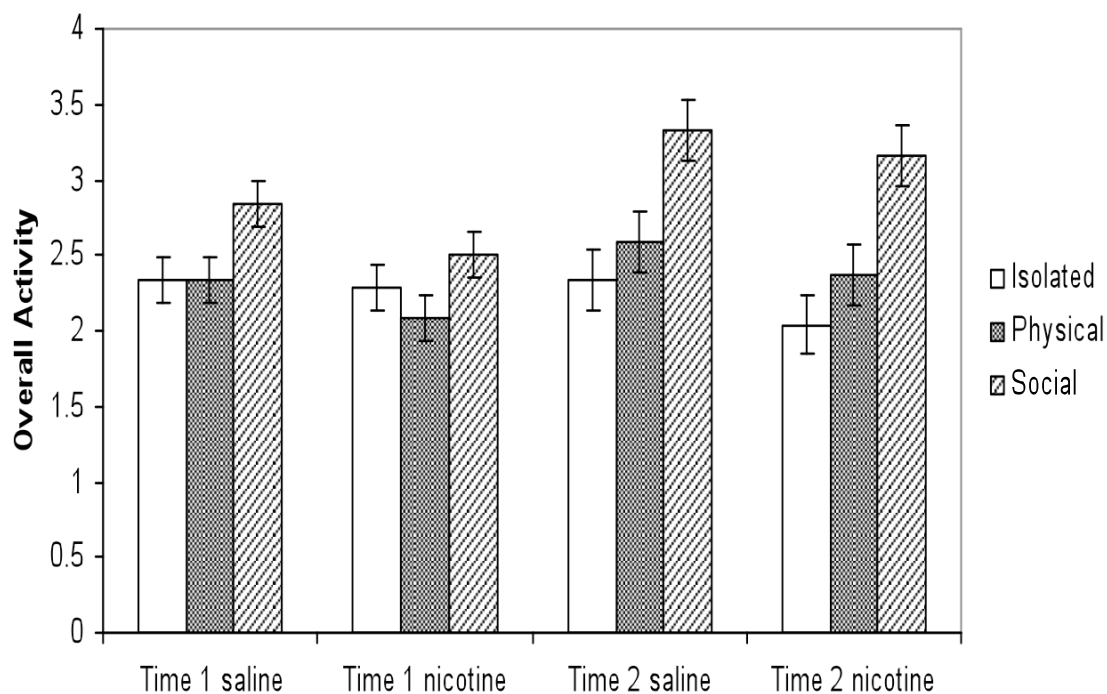


Figure 29. Mean overall activity within the home cage ($\pm$ SEM) of female, Sprague Dawley rats in three different housing conditions and two different drug conditions

Exercise (see Figure 30). A two-way ANOVA revealed no effect for housing or drug and no interactions.

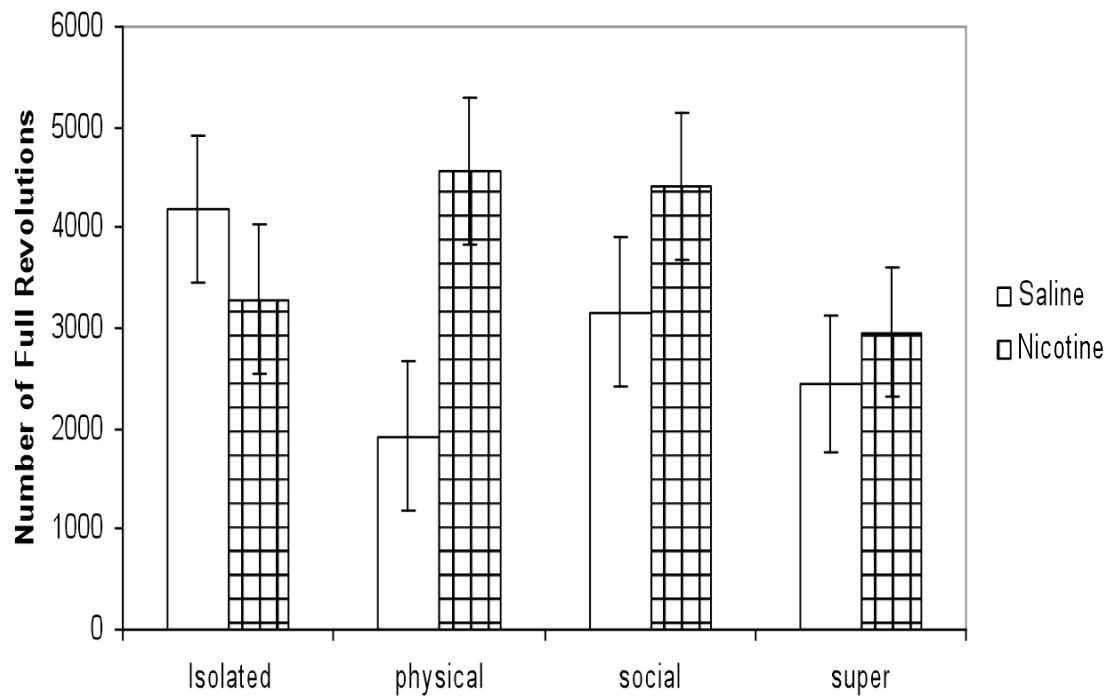


Figure 30. Mean voluntary exercise ($\pm$ SEM) of female, Sprague Dawley rats in four different housing conditions and two different drug conditions

Discussion for Experiment IIb

The purpose of this experiment was to examine the effects of environmental enrichment on nicotine cessation's effects on body weight, food consumption, and activity in female rats. Long's (2008) study examined the effects of environmental enrichment on nicotine cessation using the same dose (9 mg/kg/day), form (nicotine dihydrochloride), and dependent variables of body weight, food consumption, open field activity, home cage activity, and voluntary exercise to determine the effects of various housing conditions on nicotine cessation in male rats.

Long (2008) reported that environmental enrichment had no effects on body weight, had no effects on food consumption, decreased open field activity

(especially in the super-enriched), and that specifically the physically-enriched increased voluntary exercise whereas the super-enriched decreased voluntary exercise. Long (2008) found that nicotine cessation decreased body weight gain, had no effects on food consumption, and continued to increase voluntary exercise. Long (2008) found that nicotine cessation decreased open field vertical activity in the non-enriched and increased open field vertical activity in the super-enriched.

Similar to Long (2008), the present study found that environmental enrichment during the post-drug phase had no effects on body weight and that super-enrichment decreased open field activity. Unlike the previous work, super-enrichment decreased food consumption and non-enriched housing increased food consumption. Environmental enrichment increased home cage activity but had no effects on voluntary exercise. Also unlike Long (2008), the present study found that nicotine cessation increased body weight gain, increased food consumption, decreased horizontal and center time activity in open field activity, and had no effects on voluntary exercise (see Table 3).

IIB. Nicotine Cessation		
	Males (Long, 2008)	Females
Body weight	↓	↑
Food consumption	↔	↑
Open Field Activity	↔	↓
Home cage activity	N/A	↔
Exercise	↑	↔

Table 3. Comparison of the effects of nicotine cessation in male and female Sprague Dawley rats.

Environmental enrichment had effects on food consumption, open field activity, and home cage activity, but no longer had effects on body weight and voluntary exercise. Nicotine cessation's effects reversed the effect of nicotine on body weight and increased food consumption. However, the effects of nicotine persisted for open field activity. It appears that environmental enrichment and nicotine cessation may act independently.

General Discussion

The present experiments, with regard to environmental enrichment, replicate several findings from previous research and add new findings. The present experiments replicated past findings that environmental enrichment attenuates body weight gain (Tomchesson, 2006; Shafer, 2006; Long, 2008) and decreases open field activity (Elliott & Grunberg, 2005; Elliott, 2004; Tomchesson, 2006; Shafer, 2006; Long, 2008). The present experiments replicated past findings that environmental enrichment decreases food consumption (Tomchesson, 2006; Shafer, 2006) but differed from Long's (2008) finding that food consumption increased. The finding that environmental enrichment increased home cage activity replicated the findings by Tomchesson (2006), Shafer (2006), and Elliott (2004), but differed from Long's (2008) finding that home cage activity decreased. The present experiments did not replicate Long's (2008) findings that environmental enrichment increased voluntary exercise.

The present experiments, with regard to nicotine administration and nicotine cessation, replicated findings from previous research and add new

findings to current research. The present study replicated the finding that nicotine attenuates body weight (Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989; Long, 2008). The present study did not find a decrease in food consumption with nicotine administration that has been reported previously (Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989; Long, 2008). The present study replicated Long's (2008) finding that nicotine decreased open field activity but differed from the findings that nicotine increased open field activity by Faraday, Elliott, and Grunberg (2001) and Grunberg & Bowen (1985). It is important to note that this difference may be the result of the route of administration used in the different studies, such that acute injections of nicotine generally produce behavioral sensitization (increase behavior), whereas chronic administration of nicotine may decrease behavior over time.

The present study found that nicotine did not affect home cage activity and also found that nicotine had no effect on exercise, unlike Long (2008) who found that nicotine increased exercise.

The present study replicated the finding that nicotine cessation increases body weight (e.g., Grunberg, Bowen, & Winders, 1986; Perry, 2007), but differed from Long's (2008) finding that nicotine cessation continued to attenuate body weight gain. The present study replicated the finding that nicotine cessation increases food consumption (e.g., Winders & Grunberg, 1989), but differed from Long's (2008) finding that food consumption was not affected. The present study replicated Perry's (2007) finding that nicotine cessation decreases open field

activity, but differed from Long's (2008) finding that open field was not affected.

The present study found that nicotine cessation did not affect home cage activity and had no effects on exercise, unlike Long (2008) who found that nicotine cessation continued to increase exercise.

The present study found that both chronic nicotine administration and chronic nicotine cessation appear to decrease open field activity. It seems unusual that both nicotine administration and nicotine cessation would give rise to the same effect, and this finding deserves further research.

I. Environmental Enrichment		
	Males (Long, 2008)	Females
Body weight	↓	↓
Food consumption	↑	↓
Open Field Activity	↓	↓
Home cage activity	↓	↑
Exercise	↑	↔
IIA. Nicotine		
Body weight	↓	↓
Food consumption	↓	↔
Open Field Activity	↓	↓
Home cage activity	N/A	↔
Exercise	↑	↔
IIB. Nicotine Cessation		
Body weight	↓	↑
Food consumption	↔	↑
Open Field Activity	↔	↓
Home cage activity	N/A	↔
Exercise	↑	↔

Table 4. Comparison of the effects of environmental enrichment, nicotine administration, and nicotine cessation in male and female Sprague Dawley rats.

Several conclusions can be reached from these three experiments.

Environmental enrichment's effects on body weight cannot be fully accounted for by food consumption and activity. Enrichment, specifically super-enrichment, resulted in overall attenuated body weight gain. Super-enrichment, however, had minimal effects on food consumption and greatly decreased open field activity, had minimal effects on voluntary exercise, and greatly increased home cage activity. The attenuated weight gain, therefore, cannot be explained by observed difference in food consumption and overall activity, but may be the result of increased home cage activity or everyday activities (e.g. cleaning the house, playing games, etc.).

Another conclusion that can be reached from these experiments is that voluntary exercise provides a valuable measure of activity that is different from both home cage activity and open field activity. The most interesting findings from voluntary exercise were that there were no differences between enriched environments and non-enriched environments while there were differences in open field activity and home cage activity. These findings, in addition to the home cage and open field activity findings, indicate that different forms of enrichment affect different types of activity in different ways. No overall conclusions can be made on the effects of enrichment on activity. It is clear that one type of physical activity does not generalize to other forms of physical activity.

Chronic nicotine decreased body weight gain and open field activity, but did not affect food consumption. Cessation of chronic nicotine increased body

weight and food consumption increased. These findings suggest that stopping nicotine, once started, detrimentally affects body weight, food consumption, and some levels of activity among females.

Environmental enrichment appears to alter some of chronic nicotine's effects on voluntary exercise, but not open field activity. Environmental enrichment interacted with nicotine such that voluntary activity increased in the physically-enriched only and upon cessation the differences between housing groups disappeared.

The last overall conclusion concerns enrichment itself. Previously, it seemed that a positive linear relationship existed between enrichment and performance on a variety of tasks, such that the greater the enrichment, the greater the performance. Long (2008) suggested that the relationship may instead be an inverse U-shaped curve, similar to the Yerkes-Dodson principle where too much enrichment may be detrimental to performance. However, the present experiments did not find either a simple linear or curvilinear relationship, and it may suggest that the relationship between environment and health-related behaviors and outcomes are different for males and females, and that the relationship may depend on the specific outcome or behavior measured. For example, it appears that although super-enrichment has beneficial effects on body weight and learning (inferred from faster habituation in the open field chamber), it does not increase voluntary exercise in females. Therefore, when implementing plans for decreasing body weight in females, it is particularly important to target each specific behavior.

Limitations

This project has several limitations. The method of nicotine administration is not a perfect substitute for cigarette smoking because it only examines the addictive component of cigarette smoking, nicotine. Some of the thousands of other chemicals, not examined in this study, involved in cigarette smoking may impact lung function and physical activity. Although the chronic flow of nicotine better approximates the levels of nicotine found in smokers than daily acute injections of nicotine, it does not capture the daily fluctuations (or “boli”) in smokers. It is important to consider that chronic administration of nicotine does not accurately model the behavior of human cigarette smoking, and chronic administration (delivered 24 hours a day) may disrupt behaviors such as sleep and, therefore, may have impacted the effects found in the present study on activity. In addition, cigarette smokers control the administration of nicotine, whereas the continuous administration in this experiment was not controlled by the rats. Controllability may alter the effects of nicotine as well as stress, and these effects may alter activity, food consumption, and body weight. The home cage activity measures were limited to short observations once or twice a week, which may not have been enough to capture the level of home cage activity. Also, the home cage activity measures were not possible to use on the super-enriched condition once the drug phase began because of difficulty identifying individual rats and the inability to distinguish among the nicotine and saline rats. This limitation is critical because it may be that the beneficial effect of super-

enrichment on body weight may be the result solely of an increase in home cage activity. The voluntary exercise measure also was limited because the socially-housed rats were separated from their cage mates during the measure. The socially-enriched female rats may have experienced stress when they were separated, whereas the other housing conditions without a social component may not have experienced the same level of stress. Like Long (2008), voluntary exercise was measured rather than forced exercise, so the full impact of nicotine and environmental enrichment's effects on exercise may not have been captured.

Clinical Implications

This project supports the view that the environment can influence health risks and behaviors. Although the focus of health risk behaviors often rests on the individual, the environment has a large impact on engaging in health risk behaviors. If the present findings hold true for humans, then attempts to change health risk behaviors cannot focus solely on the individual but should also include their environment. With regard to body weight, the environment in the United States is surrounded with high calorie foods, an increase in automation, and a decrease in social interactions because of technological advances in communication such as cellular devices and the Internet. The environment is a crucial aspect that must be examined with health risk behaviors.

Long (2008) suggested that moderate amounts of physical enrichment in the environment may lead to increased voluntary exercise in males; however, this does not appear to be the case for females. Large amounts of physical and

social enrichment in the environment may lead to decreased body weight in females by increasing home cage activity rather than voluntary exercise, and this finding may be particularly relevant for adolescent females. With regard to females, the focus of increasing voluntary exercise, through gyms and workout programs, may be less effective than focusing on increasing everyday activities such as house chores, gardening, shopping, or playing games with others. Because environmental influences are contributing to the women's health problems in the United States, the environment needs to be considered.

In addition, nicotine cessation increases body weight and food consumption in females, which poses a difficult situation for females who do not want to gain weight but who want to quit smoking to improve health. Nicotine cessation programs not only need to focus on the cessation of nicotine, but also need to include treatments or interventions to target weight gain and changes in eating behaviors, especially for females.

Future Directions

Future directions include addressing some of the limitations of this study. Using a 24-hour video monitoring system to capture home cage activity would avoid the limitations of limited observation times and would capture a more complete view of the effects of environmental enrichment and nicotine on home cage activity. Also, developing a clearer identification system for rats that are socially housed, such as having the hair dyed into distinctly different colors, may allow home cage activity observations for the super-enriched conditions. Placing exercise wheels into the home cage or placing socially-housed rats into exercise

wheels together, may remove the limitation of having to separate socially-housed rats, but will surely introduce other complications. Measuring other forms of exercise such as forced exercise may provide more insight into how enrichment and nicotine affect activity behaviors and may provide insight into activity motivation. Another future direction is to have a study that includes both males and females so that more accurate comparisons can be made without confounds of time, differences in experimenters, etc. Another study including different strains of rats should be conducted to see if there are genetic differences. It also may be important to conduct a study using different dosages and forms of nicotine to see if there are differences in the effects of enrichment.

Extensions of this study are limited largely by feasibility, costs, and methodological difficulties. For example, a human study would not have the same level of experimental control even though it would have greater face validity and the ability to take into account psychosocial aspects of the human experience. The psychosocial variables would increase the number of variables and would limit the interpretations that could be made. The difficulties in interpretation and experimental control would outweigh the benefits from completing a similar study in humans.

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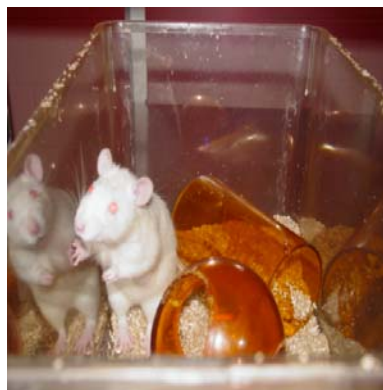
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Appendix A: Housing Pictures



Non-enriched housing



Physically-enriched housing



Socially-enriched housing



Super-enriched housing

Appendix B: Home Cage Activity Rating Form

Home Cage Activity (1 Minute Observations)

Condition: _____ Rater Initials: _____

Circle a number between 1 and 7.

	1	2	3	4	5	6	7
Number of Animals Moving	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/
	None	1-3	4-6	7-9	10-12	13-15	16

	1	2	3	4	5	6	7
Amount of Activity for Majority of Group Members	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/
	None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activity	Continuous High Activity

	1	2	3	4	5	6	7
Level of Activity	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/	/-----/
	None	Almost No Effort	Low Effort	Some Effort	Moderate Effort	Intermittent High Effort	Continuous High Effort

Indicate the type of activity and the number of animals engaged in each type of activity:

w/ Physical Object	Social Interaction	Combined P & S	Alone
_____	_____	_____	_____

Description/Comments: _____

Appendix C: Home Cage Activity Rating Form

Home Cage Activity – Version II (HCA-II)

Directions: Complete Parts A and B for each condition TWO times.

Time 1 (first 30 sec interval)

A. Level of Activity

1 2 3 4 5 6 7

None Some low Cnst low Some mod Cnst mod Some high Cnst high

Enter subject # and activity rating for each subject in the group. Rating below should correspond to arrangement on the housing rack.

For example: (Subject) # 404: (Rating) 4.

# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____
# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____

B. Record the number of subjects in this condition that are engaged in the following behaviors at the end of the observation period.

Eating	Grooming	Awake/not moving	Moving HZ	Rearing	Sleeping

Time 2 (second 30 sec interval)

A.

# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____
# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____

B.

Eating	Grooming	Awake/not moving	Moving HZ	Rearing	Sleeping

Appendix D: Experiment I Tables

Table 1 - Body Weight Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	3.32 (3, 45)	<0.05
	Time	2861.98 (1.391, 62.599)	< 0.001
	Housing x Time	1.102 (4.173, 62.599)	0.365

Table 2 - Body Weight ANOVAs			
Day (All Animals)	Effect	F value (df)	P value
1	Housing	2.102 (3, 47)	0.113
4	Housing	7.99 (3, 47)	< 0.001
7	Housing	8.502 (3, 47)	< 0.001
10	Housing	5.348 (3, 47)	< 0.01
14	Housing	4.995 (3, 47)	< 0.01
17	Housing	3.279 (3, 47)	< 0.01
21	Housing	1.868 (3, 47)	0.148
24	Housing	2.388 (3, 47)	0.081
28	Housing	2.469 (3, 47)	0.073
31	Housing	2.053 (3, 47)	0.119
35	Housing	2.439 (3, 47)	0.077

Table 3 - Food Consumption Repeated Measures ANCOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	3.047 (3, 32)	< 0.05
	Time	3.250 (5.527, 176.856)	< 0.001
	Housing x Time	2.625 (16.58, 176.856)	0.001

Table 4 - Food Consumption ANOVA			
Day (All Animals)	Effect	F value (df)	P value
3	Housing	6.008 (3, 47)	0.001
Food Consumption ANCOVAs (Day 3 as Covariate)			
Day (All Animals)	Effect	F value (df)	P value
5	Housing	10.077 (3, 40)	< 0.001
7	Housing	4.555 (3, 40)	< 0.01
9	Housing	14.522 (3, 46)	< 0.001
11	Housing	7.680 (3, 46)	< 0.001
13	Housing	5.072 (3, 46)	< 0.01
15	Housing	4.759 (3, 46)	< 0.01
17	Housing	6.333 (3, 46)	0.001
19	Housing	4.165 (3, 46)	< 0.05
21	Housing	3.288 (3, 46)	< 0.05
23	Housing	1.128 (3, 46)	0.347
25	Housing	0.876 (3, 39)	0.462
27	Housing	7.225 (3, 39)	0.001
29	Housing	2.183 (3, 46)	0.103
31	Housing	1.005 (3, 46)	0.399
33	Housing	4.687 (3, 46)	< 0.01

Table 5 – Open Field Activity Horizontal Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	14.229 (3, 39)	< 0.001
	Time	0.043 (1, 39)	0.836
	Housing x Time	1.660 (3, 39)	0.191

Table 6 – Open Field Activity Vertical Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	7.024 (3, 39)	0.001
	Time	12.594 (1, 39)	0.001
	Housing x Time	0.062 (3, 39)	0.979

Table 7 – Open Field Activity Center Time Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	4.051 (3, 39)	< 0.05
	Time	3.070 (1, 39)	0.088
	Housing x Time	1.529 (3, 39)	0.222

Table 8 – Open Field Activity First Within Session Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	20.583 (3, 39)	< 0.001
	Time	103.197 (7.055, 275.149)	< 0.001
	Housing x Time	1.673 (21.165, 275.149)	< 0.05

Table 9 - Open Field Activity Second Within Session Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	7.396 (3, 47)	< 0.001
	Time	125.568 (6.787, 319.004)	< 0.001
	Housing x Time	2.148 (20.362, 319.004)	< 0.01

Table 10 – Home Cage Activity 1 Number of Animals Moving Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	1.987 (3, 16)	0.157
	Time	2.041 (3, 48)	0.121
	Housing x Time	0.441 (9, 48)	0.906

Table 11 – Home Cage Activity 1 Amount of Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	10.237 (3, 16)	0.001
	Time	2.515 (2.101, 33.619)	0.094
	Housing x Time	0.381 (6.304, 33.619)	0.893

Table 12 – Home Cage Activity 1 Effort of Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	13.235 (3, 16)	< 0.001
	Time	1.126 (3, 48)	0.348
	Housing x Time	0.426 (9, 48)	0.915

Table 13 – Home Cage Activity 2 Overall Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	0.627 (2, 33)	0.541
	Time	15.982 (3.022, 99.727)	< 0.001
	Housing x Time	1.648 (6.044, 99.727)	0.141

Table 14 - Exercise Repeated Measures ANOVAs			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	0.166 (3, 46)	0.919
	Time	12.298 (1, 46)	0.001
	Housing x Time	1.648 (3, 46)	0.052

Appendix E: Experiment IIa Drug Phase Tables

Table 1 - Body Weight Repeated Measures ANCOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	3.296 (3, 27)	< 0.05
	Drug	18.581 (1, 27)	< 0.001
	Time	0.441 (1.824, 49.239)	0.628
	Housing x Drug	1.457 (3, 27)	0.139
	Housing x Time	1.121 (5.471, 49.239)	0.363
	Drug x Time	4.776 (1.824, 49.239)	< 0.05
	Time x Housing x Drug	0.493 (5.471, 49.239)	0.795

Table 2 - Food Consumption Repeated Measures ANCOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	1.836 (3, 37)	0.158
	Drug	0.596 (1, 37)	0.446
	Time	8.668 (2.592, 1703.382)	< 0.001
	Housing x Drug	2.307 (3, 37)	0.092
	Housing x Time	3.365 (7.775, 1703.382)	0.001
	Drug x Time	6.582 (2.592, 1703.382)	0.001
	Time x Housing x Drug	3.954 (7.775, 1703.382)	0.001

Table 3 - Food Consumption ANOVAs			
Day (All Animals)	Effect	F value (df)	P value
4	Drug	14.504 (1, 42)	< 0.001
	Housing	0.239 (3, 42)	0.868
	Drug x Housing	3.625 (3, 42)	< 0.05
6	Drug	1.301 (1, 41)	0.261
	Housing	2.667 (3, 41)	0.060
	Drug x Housing	0.320 (3, 41)	0.811
8	Drug	3.948 (1, 37)	0.054
	Housing	7.696 (3, 37)	< 0.001
	Drug x Housing	3.749 (3, 37)	< 0.05
10	Drug	0.079 (1, 38)	0.780
	Housing	0.973 (3, 38)	0.416
	Drug x Housing	1.818 (3, 38)	0.160
14	Drug	4.628 (1, 39)	< 0.05
	Housing	2.767 (3, 39)	0.055
	Drug x Housing	6.152 (3, 39)	< 0.01

Table 4 - Open Field Horizontal Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	19.609 (3, 43)	< 0.001
	Drug	0.213 (1, 43)	0.647
	Time	0.719 (1, 43)	0.401
	Housing x Drug	2.036 (3, 43)	0.123
	Time x Housing	1.045 (3, 43)	0.382
	Time x Drug	5.743 (1, 43)	< 0.05
	Time x Housing x Drug	0.456 (3, 43)	0.715

Table 5 - Open Field Vertical Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	12,231 (3, 43)	< 0.001
	Drug	1.008 (1, 43)	0.321
	Time	8.096 (1, 43)	< 0.001
	Housing x Drug	0.809 (3, 43)	0.496
	Time x Housing	0.493 (3, 43)	0.751
	Time x Drug	5.643 (1, 43)	< 0.05
	Time x Housing x Drug	1.472 (3, 43)	0.236

Table 6 - Open Field Center Time Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	5.524 (3, 43)	< 0.01
	Drug	0.111 (1, 43)	0.741
	Time	0.043 (1, 43)	0.837
	Housing x Drug	0.188 (3, 43)	0.904
	Time x Housing	0.135 (3, 43)	0.939
	Time x Drug	5.057 (1, 43)	< 0.05
	Time x Housing x Drug	3.667 (3, 43)	< 0.05

Table 7 - Open Field Activity First Within Session Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	12,087 (3, 43)	< 0.001
	Drug	1.667 (1, 43)	0.203
	Time	97.161 (6.314, 271.522)	< 0.001
	Housing x Drug	0.991 (3, 43)	0.406
	Time x Housing	1.355 (18.943, 271.522)	0.150
	Time x Drug	0.992 (6.314, 271.522)	0.433
	Time x Housing x Drug	0.834 (18.943, 271.522)	0.666

Table 8 - Open Field Week 7 Activity Second Within Session Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	23.229 (3, 43)	< 0.001
	Drug	0.597 (1, 43)	0.444
	Time	142.728 (6.757, 290.571)	< 0.001
	Housing x Drug	3.097 (3, 43)	< 0.05
	Time x Housing	2.673 (20.272, 290.571)	< 0.001
	Time x Drug	1.403 (6.757, 290.571)	0.206
	Time x Housing x Drug	0.971 (20.272, 290.571)	0.498

Table 9 – Home Cage Activity 2 Overall Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	2.607 (2, 30)	0.090
	Drug	0.428 (1, 30)	0.518
	Housing x Drug	0.622 (2, 30)	< 0.01
	Time x Housing	3.137 (2, 30)	0.058
	Time x Drug	4.101 (1, 30)	0.052
	Time x Housing x Drug	0.776 (2, 30)	0.469

Table 10 – Exercise Two-Way ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	0.311 (3,43)	0.818
	Drug	0.344 (1, 43)	0.561
	Housing x Drug	3.634 (3, 43)	< 0.05

Appendix F: Experiment IIb Post-Drug Phase Tables

Table 1 - Body Weight Repeated Measures ANCOVAs			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	1.179 (3, 26)	0.120
	Drug	7.003 (1, 26)	< 0.05
	Time	0.425 (2, 52)	0.656
	Housing x Drug	0.464 (3, 26)	0.051
	Housing x Time	0.937 (6, 52)	0.477
	Drug x Time	1.906 (2, 52)	0.159
	Time x Housing x Drug	0.678 (6, 52)	0.668

Table 2 - Food Consumption Repeated Measures ANCOVAs			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	3.642 (3, 30)	< 0.05
	Drug	30.607 (1, 30)	< 0.001
	Time	0.195 (2.442, 73.259)	0.864
	Housing x Drug	0.771 (2, 30)	0.499
	Housing x Time	4.200 (7.326, 73.259)	0.001
	Drug x Time	2.672 (2.442, 73.259)	0.065
	Time x Housing x Drug	1.435 (4.884, 73.259)	0.223

Table 3 - Food Consumption ANOVAs			
Day (All Animals)	Effect	F value (df)	P value
4	Drug	0.003 (1, 39)	0.955
	Housing	1.851 (3, 39)	0.154
	Drug x Housing	0.970 (3, 39)	0.416
6	Drug	4.089 (1, 39)	0.05
	Housing	3.228 (3, 39)	< 0.05
	Drug x Housing	1.736 (3, 39)	0.175
10	Drug	5.272 (1, 31)	0.150
	Housing	0.893 (3, 31)	0.566
	Drug x Housing	1.534 (3, 31)	0.232
12	Drug	3.267 (1, 37)	0.079
	Housing	2.929 (3, 37)	< 0.05
	Drug x Housing	1.425 (3, 37)	0.251

Table 4 - Open Field Horizontal Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	24.799 (3, 43)	< 0.001
	Drug	9.647 (1, 43)	< 0.01
	Time	3.522 (1, 43)	0.067
	Housing x Drug	1.062 (3, 43)	0.375
	Time x Housing	1.261 (3, 43)	0.300
	Time x Drug	1.302 (1, 43)	0.260
	Time x Housing x Drug	0.388 (3, 43)	0.762

Table 5 - Open Field Vertical Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	21.352 (3, 43)	< 0.001
	Drug	0.575 (1, 43)	0.452
	Time	7.725 (1, 43)	< 0.01
	Housing x Drug	1.224 (1, 43)	0.313
	Time x Housing	0.922 (3, 43)	0.438
	Time x Drug	1.629 (1, 43)	0.209
	Time x Housing x Drug	1.121 (3, 43)	0.351

Table 6 - Open Field Center Time Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	3.884 (3, 43)	< 0.05
	Drug	9.074 (1, 43)	< 0.01
	Time	2.413 (1, 43)	0.128
	Housing x Drug	0.167 (3, 43)	0.918
	Time x Housing	0.769 (3, 43)	0.518
	Time x Drug	0.004 (1, 43)	0.952
	Time x Housing x Drug	0.399 (3, 43)	0.755

Table 7 - Open Field Activity First Within Session Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	12.757 (3, 43)	< 0.001
	Drug	3.441 (1, 43)	0.070
	Time	153.375 (7.039, 302.670)	< 0.001
	Housing x Drug	0.857 (3, 43)	0.471
	Time x Housing	3.634 (21.117, 302.670)	< 0.001
	Time x Drug	1.494 (7.039, 302.670)	0.168
	Time x Housing x Drug	0.847 (21.117, 302.670)	0.661

Table 8 - Open Field Activity Second Within Session Repeated Measures ANOVAs			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	38.635 (3, 43)	< 0.001
	Drug	17.329 (1, 43)	< 0.001
	Time	134.615 (7.229, 310.844)	< 0.001
	Housing x Drug	1.450 (3, 43)	0.092
	Time x Housing	1.671 (21.687, 310.844)	< 0.05
	Time x Drug	1.249 (7.229, 310.844)	0.274
	Time x Housing x Drug	1.146 (21.687, 310.844)	0.297

Table 9 – Home Cage Activity 2 Overall Activity Repeated Measures ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	14.124 (2, 30)	< 0.001
	Drug	3.316 (1, 30)	0.079
	Time	8.356 (1, 30)	< 0.01
	Housing x Drug	0.045 (2, 30)	0.956
	Housing x Time	5.941 (2, 30)	< 0.01
	Drug x Time	0.007 (1, 30)	0.935
	Time x Housing x Drug	0.539 (2, 30)	0.589

Table 10 - Exercise Two-Way ANOVA			
Experimental Group	Effect	F value (df)	P value
All Animals	Housing	1.071 (3, 43)	0.371
	Drug	2.959 (1, 43)	0.093
	Housing x Drug	2.006 (3, 43)	0.127