April 2006

Effects of Stress and Nicotine on ADHD Rat Models

Krisha S. Murthy
Worcester Polytechnic Institute

Project Number: BME-JMS-0602

Effects of Stress and Nicotine on ADHD Rat Models

A Major Qualifying Report

Submitted to the Faculty

Of the

Worcester Polytechnic Institute

In Partial Fulfillment of the Requirements for the

Degree of Bachelor of Science

By

Krisha S. Murthy

Date: April 27, 2006

Approved:

_____

Professor John Sullivan, Major Advisor

_____

Dr. Jean King, Co-Advisor

Table of Contents

Acknowledgements

Thank you to Professor John Sullivan and Dr. Jean King for their advising and support, and also to Praveen Kulkarni, Tim Garelick, Tara Messenger and Wei Chen for their time and help.

Table of Figures

Abstract

While the percentage of smokers has decreased among the common population, the percentage of smokers suffering from attention-deficit hyperactivity disorder has almost doubled. Psychological stressors may increase the desire to smoke among smokers, as well as lead to relapse after quitting. The effects of stress on both conditions (ADHD and nicotine addiction) are a topic that deserves more investigation. This simultaneous presence of two or more conditions in a single individual is called comorbidity. When more than one condition co-occurs, the consequences of the conditions may affect a person differently than when the condition occurs in singularly.

This coexistence of conditions can be investigated using animal models. The genetics of a typical laboratory rat can be altered to simulate an ADHD sufferer, while an unaltered rat was used as a non-ADHD sufferer. A stress was applied, nicotine was administered, and behavioral sensitization was measured by locomotion. Once the rats were sensitized to the nicotine, they were imaged using functional magnetic resonance imaging to provide a picture of the effects the combination of ADHD, nicotine, and stress on the brain. This should corroborate the behavioral data gathered from locomotion.

It is hypothesized that nicotine sensitization will be greater in the ADHD model than in the non-ADHD model. With the presence of stress, this sensitization will occur still greater. The addition of stress to the situation is predicted to amplify sensitization. This means the addition of stress should increase the amount of movement and increase sensitization rate. The data showed a greater nicotine sensitization for the ADHD model compared to the non-ADHD model, the control. However, sensitization was not amplified when stress was coupled to nicotine for both models. The fMRI results corroborated these findings.

Introduction

While the percentage of smokers has decreased, the percentage of smokers among those suffering from attention-deficit hyperactivity disorder (ADHD) has almost doubled. Psychological stressors may increase the desire to smoke among smokers, as well as lead to relapse after quitting. The property that causes this addiction to smoking and effects on the brain is found in the nicotine contained in cigarettes. The effects of stress on nicotine addiction in ADHD sufferers are a topic that deserves more investigation. This coexistence of two or more conditions in a single individual is called comorbidity. When more than one condition co-occurs, the consequences of the conditions may affect a person differently than when the conditions occur singularly.

Background

Attention-deficit/hyperactivity disorder, better known as ADHD, is a disorder with many possible contributing factors including strong genetic roots.[1] ADHD is physically demonstrated in a person by daydreaming, distractibility, and difficulty focusing on a single task for a prolonged period in the attention deficit component; the hyperactivity component is demonstrated by fidgeting, excessive talking, and restlessness. The symptoms of ADHD have been shown to cause accidents, cause strain in personal relationships, and disrupt the environment through interruptions and inappropriate behavior, not just for those with the disorder, but everyone. [2]

Studies on whole families have shown that heredity plays a major role in the onset of ADHD. Family studies have demonstrated a two to eight times increase in the risk for ADHD in parents and siblings of children with ADHD as can be seen in the chart below[3]:

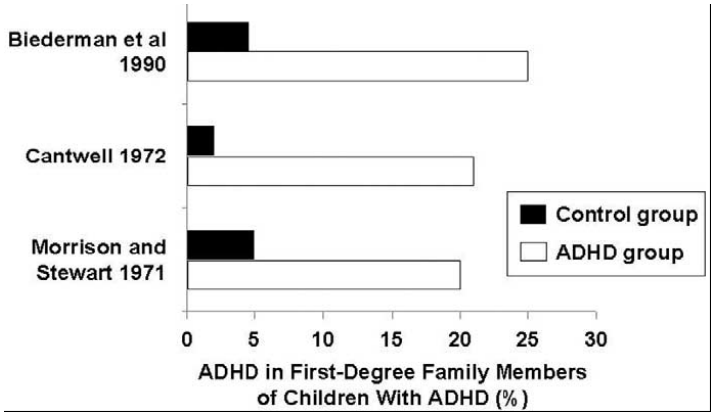

Figure 1: This graph shows the high probability that a parent with ADHD will pass that disorder onto their offspring versus a parent without ADHD in three different studies.

While conducting studies that involve the control of drug addiction, there have been families involved that have shown that genes can also contribute to the

vulnerability of addictive diseases/disorders. This was first shown with alcoholism, which is specifically enhanced by an aldehyde dehydrogenase 2 genotype. Since then, there have been genes associated with the opioid system, serotonergenic system and more. [4] These and other data offer the suggestion that persistent ADHD might be a useful phenotype for molecular genetic studies such as this study to determine effects of stress on an ADHD model, i.e. the spontaneously hypertensive rats (SHR), to determine any change in patterns of addiction. Different strains, or different rat models, are able to show the differences in the cellular and molecular responses under different conditions.

Stress is defined as a medical term for strong external stimuli, both physiological and psychological, which can cause a physiological response called the general adaptation syndrome. This general adaptation syndrome causes heart rate, blood pressure and respiration to rise and the "flight or fight" mechanism to deploy.[5]

Stress has been shown to be one of the most common features reported to cause a relapse in smoking.[6] After quitting, the most common side effects are anxiety, irritability, depression, and craving, which are intensified by stress. Stress has also been shown to increase smoking among current smokers and speeds up the progression of a possible relapse to smokers after quitting. The exact causes of these events due to stress that impact a relapse are not positively known. However, recent advancements in scientific research suggest that many neuroendocrine, psychosocial, and biobehavioral mechanisms all act with stress effects on relapse[7].

When a smoker stops smoking, he/she experiences symptoms like irritability, craving, anxiety, depression, restlessness, and difficulty concentrating. These symptoms begin almost immediately after the nicotine is taken away, within the first twenty-four hours. These effects of the lack of smoking on mood may be part of what causes a high level of stress, and hence could increase the desire to smoke[8].

The effects that acute doses of nicotine have on the hypothalamic–pituitary adrenocortical (HPA) axis have been documented previously and are shown to be a stimulant. Following nicotine administration, dose-dependent increases in brain activity have been noted in regions involved in emotion regulation and HPA responses to stress.[9] Exposure to stress also activates the HPA axis, and the activation or lack of activation impacts addiction.[10] Nicotine also activates the HPA axis, and increased HPA activity has been linked to attenuated CNS nicotinic receptor sensitivity.[11] This connection is one reason research involving the differences in stress response between smokers and non-smokers is necessary. In previous animal models, it was shown that in previous animal models stressors have influenced the reinforcing effects of a drug of abuse.[12]

A susceptibility to developing a drug addiction is influenced by many factors including ones from genetics and environment. However, the effects directly from the drug also affect the progression of addiction, as well as the tendency to quit and relapse after being clean for a long time period. Rates of cigarette smoking are the highest among patients with psychotic and substance-use disorders, but it is also high for depression, anxiety, and personality

disorders. Both children and adults with ADHD are significantly more likely to smoke than people without the disorder. In a laboratory environment, nicotine has been previously shown to reduce the symptoms of ADHD because it acts as a dopamine agonist. This sort of positive reinforcement encourages smoking among these ADHD sufferers. [13]

After multiple administrations of a drug of abuse, there is an increase in psychomotor stimulant effects and positive reinforcing effects, where a psychomotor stimulant is defined as a group of drugs, including cocaine, amphetamine, methylphenidate, and ephedrine that produce an awakening effect and stimulate behavior. This set of events is defined as behavioral sensitization and occurs with repeated use of substances of abuse. Results of locomotor tests suggest long term effects of the repeated use of nicotine.[14] One study's preliminary work demonstrates that are differences in locomotor sensitization among inbred mouse strains. In other words, there are differences in locomotor sensitization among the different genetic models. Studies on these inbred strains could possible be able to clarify what genes do in response to nicotine-induced sensitization.[15]

It is believed that drug-induced behavioral sensitization is an important process in the development of a substance addiction. Repeated injections of nicotine produce gradual increases in locomotor activity in rats and mice which is considered behavioral sensitization. Nicotine-induced sensitization has been widely studied using this model, because sensitization is believed to be an important process in the development of drug addiction. The model has been in use since 1984. The repeated injections of nicotine that produce behavioral sensitization result in an increase of extracellular dopamine release in the nucleus accumbens and the striatum. It is likely nicotine-induced sensitization changes not only neural transmission, but also gene expression in the mesolimbic system.[16] It is important to explore these areas using functional magnetic resonance imaging (fMRI) to see the exact changes in the ADHD brain as a result of smoking compared to the changes in the brain as a result of smoking AND stress.

Because of the effects drug sensitization, specifically nicotine sensitization, as well as stress and genetics, have on the neuronal mechanisms, as well as behavioral symptoms, it appears that more research is needed on the connections. Specifically, the effect of stress on nicotine sensitization on an ADHD rat model compared to a "typical rat" model will be investigated.

Models are a tried and true method used to predict what will happen in "real life". Architects make models of buildings before they build a life size building to predict if the building will successfully stand. If the model breaks or cracks in certain places, the blueprints for the building will have to be updated. Without a model, people could be hurt using this building under unsafe conditions. This idea can also be translated into science. Drugs are tested on animal models to see the effects that the drug would have on a range of people. Devices are tested on animals to see if they perform their duty properly and comfortably. Models also allow the investigator to have control of the environment. In this case, using models will enable a prediction of what would

happen in humans under these very common human conditions in a controlled environment.

The main type of rat used in research is the Sprague-Dawley (SD) rat. By manipulating the genes in this rat, scientists have created a way to model some genetically based human conditions. To create a successful model of attention deficit and hyperactive disorder (ADHD), needed for this study, where the symptoms are all behavioral, there are multiple areas in the brain that control the behavior that need to be examined to get the precise combination of effects that make up ADHD. These symptoms must be comparative to the human symptoms of ADHD in order to be a successful model.

Animal Modeling

To create a successful animal model certain criteria must be met. Validity and reliability are the two factors on which animal models are evaluated. There are four aspects to validity: face, predictive, etiological and genetic. Face validity describes the similarity between the animal model and a specific human behavior. Although the behavior of the human behavior and the animal behavior do not need to be exactly similar, the behavior should be comparable. Predictive validity describes the extent that an animal model will allow predictions to be made about the human behavior, or how useful the model will be. Etiological validity focuses on the similarity between mechanisms that are involved in the behavior of the animal model and the human. Genetic validity is present when the genetic component in both humans and the animal model is similar. In most psychiatric disorders, there is a genetic component. The reliability refers to the stability and reproducibility of the model. [17]

For an ADHD model, the above criteria can be made more specific. For face validity, the model should mimic the fundamental behavioral characteristics of ADHD. For instance, impulsiveness should be absent initially and develop gradually over time, sustained attention-deficit should be demonstrated only when stimuli are widely spaced in time, hyperactivity should not be observed in a novel, non-threatening environment and it should also develop over time. In terms of etiological validity, an ADHD model should demonstrate the two main behavioral patterns have been shown to be major contributory factors in the origin of ADHD: altered reinforcement of novel behavior and deficient extinction of previously reinforced behavior. The model should predict aspects of ADHD behavior to show predictive validity and the model should be preferably a genetic model. [18]

The ADHD model, SHR, shows these listed behavioral characteristics of an ADHD sufferer: impaired sustained attention without obvious sensory problems, motor impulsiveness, and hyperactivity that develops over time when reinforcers are not frequent. Just like children with ADHD, SHRs display increased behavioral variability, deficient response re-engagement, and make a greater amount of error than controls. Besides adhering to behavioral criteria for an

animal model of ADHD, SHR fulfils the genetic validity statement in that it is a genetic model of ADHD bred from the WKY rats, where WKY serve as a valid control for SHR since their behavioral characteristics are similar to those of other rat strains. SHR rats were originally used as hypertension models as they age, however, while in adolescence, they show the symptoms of ADHD. WKY rats are widely used as a control for hypertension.

The theory to get this specific model of ADHD is based on the hypothesis that altered dopaminergic function doesn't adjust the nondopaminergic signal transmission correctly. A hypofunctioning mesolimbic dopamine receptor will give a behavior that shows delayed aversion, development of hyperactivity in novel situations, impulsiveness, deficient sustained attention, increased behavioral variability, and failure to reduce responses. A hypofunctioning mesocortical dopamine receptor will cause attention response deficiencies.[19] For instance, if the animal is placed in a box with an object somewhere in the box, the non-ADHD model rat will take the straightest path to the object, while the ADHD model will take detours, but eventually end up in the same place. Other attention response deficiencies include flawed orienting responses, impaired eye movements, and poor behavioral planning. A hypofunctioning nigrostriatal dopamine branch will cause impaired motor functions and a lacking implicit habit learning and memory. These deficiencies give rise to apparent developmental delay, clumsiness, and small abnormalities in sensory and motor responses when quick reactions are required. These symptoms all together will give an ADHD model that is comparative to the human standards of what ADHD is.

Three candidate dopamine genes (DRD2, DRD4, and DAT) were sequenced in SHR and WKY. No differences were found in DRD2 or DRD4 genes but a 160 blood pressure insertion was found in the non-coding region upstream of axon 3 of the DAT1 gene. This is where the differences lie, genetically, between the WKY and the SHR rats. The DAT gene has been associated with ADHD in several family studies. Alterations in DAT1 gene expression can affect dopamine uptake and reutilization. For example decreases in the expression of DAT1 will reduce reuptake and increase metabolism of dopamine. Differences in dopamine metabolism have been reported for children and adults with ADHD DOPA decarboxylase activity was found to be increased in the midbrain of children and decreased in prefrontal cortex of adults with ADHD compared to controls. Reduced DAT1 expression at a young age would reduce dopamine reuptake, thereby reducing dopamine reutilization and necessitating increased synthesis of dopamine by DOPA decarboxylase. In adults, increased expression of DAT1 might be expected to increase reuptake of dopamine, thereby reducing the need for synthesis by DOPA decarboxylase.[20]

SHR appear to have higher extracellular tonic dopamine in the nucleus accumbens shell. However, consistent with increased DAT1 expression in adult SHR striatum, extracellular dopamine levels are decreased in the caudate nucleus and d-amphetamine-stimulated release of dopamine via DAT1 is greater in SHR striatum than WKY. These findings suggest that increased expression of the DAT1 gene may reflect an attempt to compensate for increased tonic extracellular

dopamine in the nucleus accumbens shell of SHR or increased DAT1 expression may occur in an attempt to compensate for decreased function of DAT1 in adult SHR striatum.[21]

Hypertension is a confounding factor in the SHR model of ADHD. However, SHR do not develop hypertension until they are adults, from 10 to 12 weeks of age, whereas hyperactivity is observed at 3 to 4 weeks of age before they enter puberty. Analysis of their behavior revealed that locomotion mapped to chromosomes 3, 8 and 18 while hypertension exhibited multigenic complexity with both environment and genetic background as contributing factors. SHR behavior was suggested to result from an interaction between genetics and the environment, much like ADHD.[22]

In addition to behavioral and genetic similarities to ADHD, SHR exhibit brain pathology similar to ADHD. SHR brain volumes, specifically prefrontal cortex, occipital cortex, and hippocampus, are smaller than controls. MRI revealed significantly increased ventricular volume in SHR compared to WKY at 3 months of age. There are fewer neurons in these brain areas compared to WKY. Taken together, these studies point to a definitive genetic link between SHR and ADHD individuals.

Expected Results

The objective of this experiment is to determine the effects of stress and nicotine sensitization separately and jointly on an ADHD model and control models. The hypothesis states that the rats considered to be the ADHD model will sensitize to nicotine greater than non ADHD rats. With an added stress, it is expected that both sets of rats will sensitize greater still. Quantitatively, stress will make the ADHD models move a greater total distance and sensitize faster. The non ADHD models will also increase locomotion due to stress, but not to the same degree as the ADHD models.

In total, there are six different sets of rats: SD without stress, SD with stress, WKY without stress, WKY with stress, SHR without stress and SHR with stress. Each of these rats is a different model, and hence will each have different results. The expected results for the precursor (WKY) and the ADHD model (SHR) are shown, graphically.

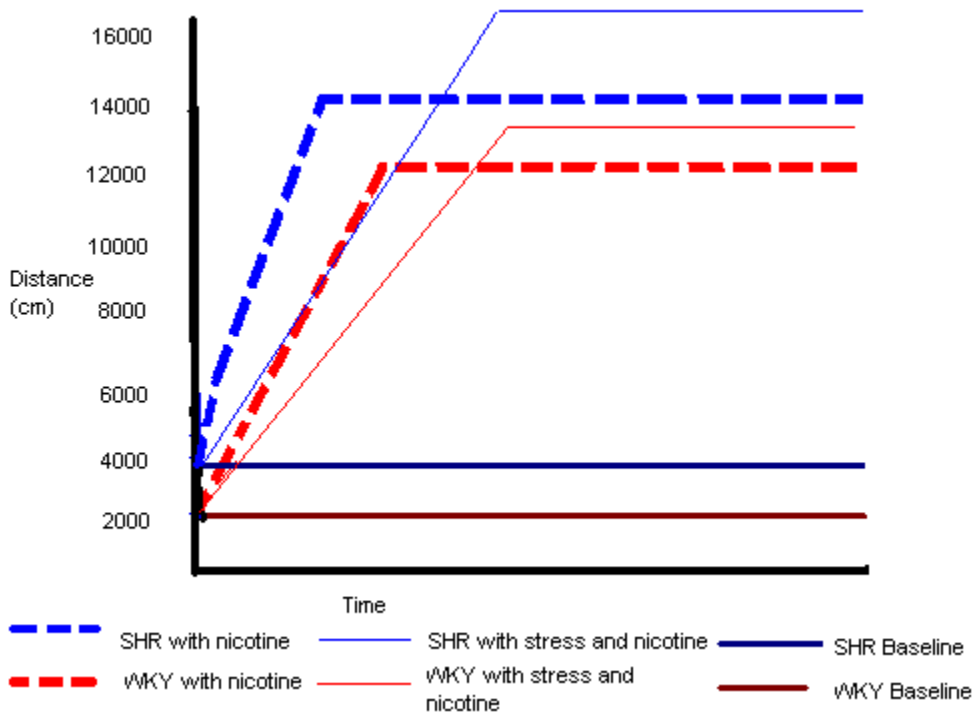

Figure 2: This figure shows the expected movement of each of the six sets of rats versus time, with the time on the x-axis and the movement on the y-axis.

Methods

Subjects

Three types of rats were used. These include a model for ADHD (SHR), the genetic precursor to the ADHD models (WKY), and the original species of rat, Sprague-Dawley, or SD. These rats were divided into two groups of each rat type, saline and nicotine. The nicotine group was then subdivided into two groups of stress and no stress, and another group was added to consider stress alone.

Equipment

Behavioral

To measure the movement of the subjects, they were each placed into a clean, black box. Above the box was a camera to track the subject. A computer is attached to this camera and contains a program called Ethovision, which was used to automatically record the activity levels (distance traveled), and then

export this raw data to an Excel sheet. This setup can be seen below in the following figure.

Figure 3: The animals were placed in the black box above to gather the behavioral data for this study.

fMRI

Functional magnetic resonance imaging was conducted using a Brucker Biospec 4.7-T/40 cm horizontal magnet equipped with a Biospec Brucker console and a 20 G/cm magnetic field gradient insert. Images will all be obtained with a BOLD-weighted multislice fast spin echo pulse sequence with the following parameters: 14 slices, 1.2 mm thick; field of view, 30 mm; 64x64 data matrix; echo train length (ETL), 16; echo spacing, 7 ms; repetition time (TR), 2108 ms; effective echo time, 7 ms.[23] To contain the animals before placing the in the magnetic coil, the y were placed into a two-part coil system.

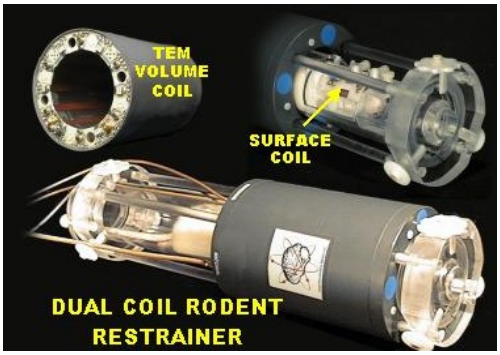

Figure 4: Animals were secured with this multi-concentric dual-coil, small animal restrainer.

Figure 5: Once the particular animal was secured, it was placed into the above magnet for fMRI.

Procedure

Specifically, the progression of events was as such for each set of rats:

Event	Days
Acclimation (to the MRI machine)	3
Habituation (acclimation to the environment and the injection)	2
Sensitization (administration of nicotine until distance traveled levels off)	5-7
Imaging	1

These values come from research from outside journals as described below.

Experimental Protocol Time Table

SHR		**WKY**		**SD**	
Nicotine (n=4)	08/05	**Nicotine (n=4)**	08/05	**Nicotine (n=4)**	06/05
Saline (n=4)	08/05	**Saline (n=4)**	08/05	**Saline (n=4)**	06/05
Nicotine+Stress (n=4)	1/06	**Nicotine+Stress (n=4)**	2/06	**Nicotine+Stress**	03/06
Stress (n=4)	3/06	**Stress (n=4)**	03/06	**Stress**	03/06

This table shows the dates of when the behavioral data was gathered.

fMRI

Functional imaging data shows high quality images of the inside of the human body. Magnetic resonance began as a tomographic imaging method for producing nuclear magnetic resonance (NMR) images of a slice through the body, where each slice has a certain thickness. A tomographic imaging method is one in which detailed x-rays of a predetermined plane section of a solid object are made while blurring out the images of other planes. The magnetic resonance image that is gathered is made up of many pixels. The intensity of a pixel is proportional to the intensity of the contents of the corresponding volume element of the object being imaged. The intensity being measured here is based on the absorption and emission of energy from the electromagnetic spectrum.[24]

Acclimation Phase

When placing a rat into an MRI machine, acclimation prepares the rat for this experience. Acclimation is when the animal is placed in a restraint device similar to the one in an MRI machine. This is because being placed in a restraint is also a stressor for rats and hence changes the physiological response. When an animal is acclimated, it becomes used to the restraint and the act of this certain restraint is no longer a stress. In one experiment to determine how to acclimate rats to an MRI machine, the animals were acclimated for eight days and the heart rate gathered on interval. The results showed that by the 3rd day of acclimation, the heart rate had dropped to normal range, showing that the animal no longer experiences the physiological act of stress.[25]

Habituation Phase

Habituation is defined as a decline of a conditioned response following a repeated exposure to the conditioned stimulus, in this case, the stimulus being a new environment. Habituation is done to prevent the response of the unwanted stimulus to concentrate on the stimulus being studied.[26] Previous to any nicotine injections, all of the rats will be habituated into the environment, as well as to a saline injection, for 30 minutes for two days. This will also allow a baseline to compare to the distances traveled after the nicotine injections begin.

It has been documented that habituation only takes one day in behavioral sensitization tests. For instance, in one study that sensitized the animals to nicotine also, all the animals were given saline on the first day, however by the second day; they were split into a “nicotine” group and a “saline” group.[27] More than one study involving behavioral sensitization had the rats being habituated for one day to acclimate to the surroundings, and the following day meant for habituation to the injection.[28,29]

Sensitization

Sensitization is defined as increase in responsiveness upon repeated exposure to a stimulus, the stimulus being nicotine. This is demonstrated by the increase in locomotion that is measured. When the locomotion quantity has leveled off, the animal has been sensitized to the stimulus, nicotine.

Drug Administration

A subcutaneous injection of .4 mg/kg nicotine was used. Previous research has shown this number to be most commonly used in subcutaneous injections.[30] Intramuscular injections and intravenous injections were eliminated because rats have limited muscle mass and the skin overlying the vessels in the adult rat is very thick, making injections difficult. Subcutaneously injected drugs will begin

their effects within 5 minutes.[31] When the nicotine solution is mixed, it is tested as to achieve a pH of 7. This is done because the pH of 7 is a neutral pH which won't cause any internal reactions when injected. Both groups of each type of rat were exposed to daily nicotine injections; however, one group (a nicotine + stress group) was also given a stress. The injections were given daily for 5 days, which personal previous research has shown to achieve sensitization in these rats. The rats were, immediately following the injection, placed into the box where the movement data was acquired for 30 minutes.

Stress Manipulation

Stress is thought to expedite the process of sensitization, although, some data has shown that this is only the case for adult female rats, not adult male rats. Each rat in the stress group was subject to a stress of an air puff for just one puff (1-2 seconds) immediately following the injection to investigate the hypothesis. This stress of an air puff was chosen because in past research, it has been observed to elicit this response in rats. The air puff has been shown to cause submaximal cardiovascular changes in rats, and to double the heart rate in Sprague-Dawley rats, as well as activate the HPA axis.[32] From personal observation, the behavior following the air puff has appeared to be an increase heart rate and freezing of the rat, meaning the rat stops all movement, although there was no quantitative measurement.

Results

Sensitization

The results of the nicotine sensitization are graphically presented below, with the standard deviation of n-1 shown. The results for saline only animals are presented first: SD, WKY and SHR, respectively. Next are the results for the nicotine only animal groups, in the same order as above. Then, shown are the results for these animals under a stress with nicotine, again in the same order, and lastly shown are the results for each set of animals under a stress alone.

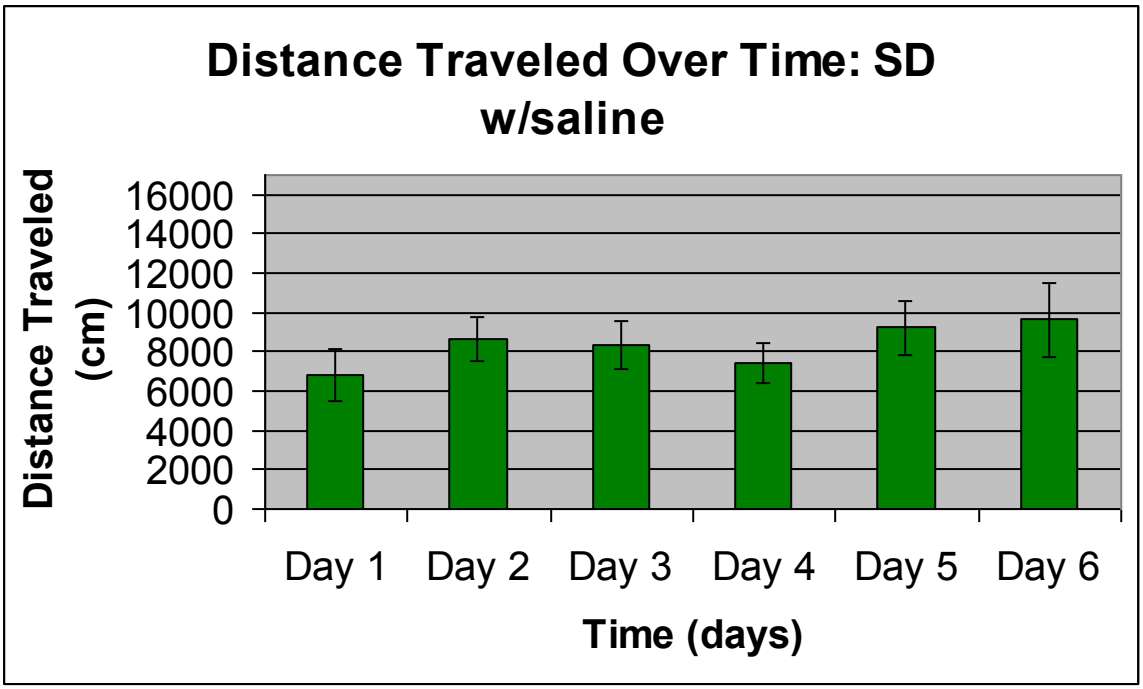

Figure 6: This figure shows the movement for an n=4 of SD animals being injected with only saline. The movement stays basically constant at an average distance of 8500 cm.

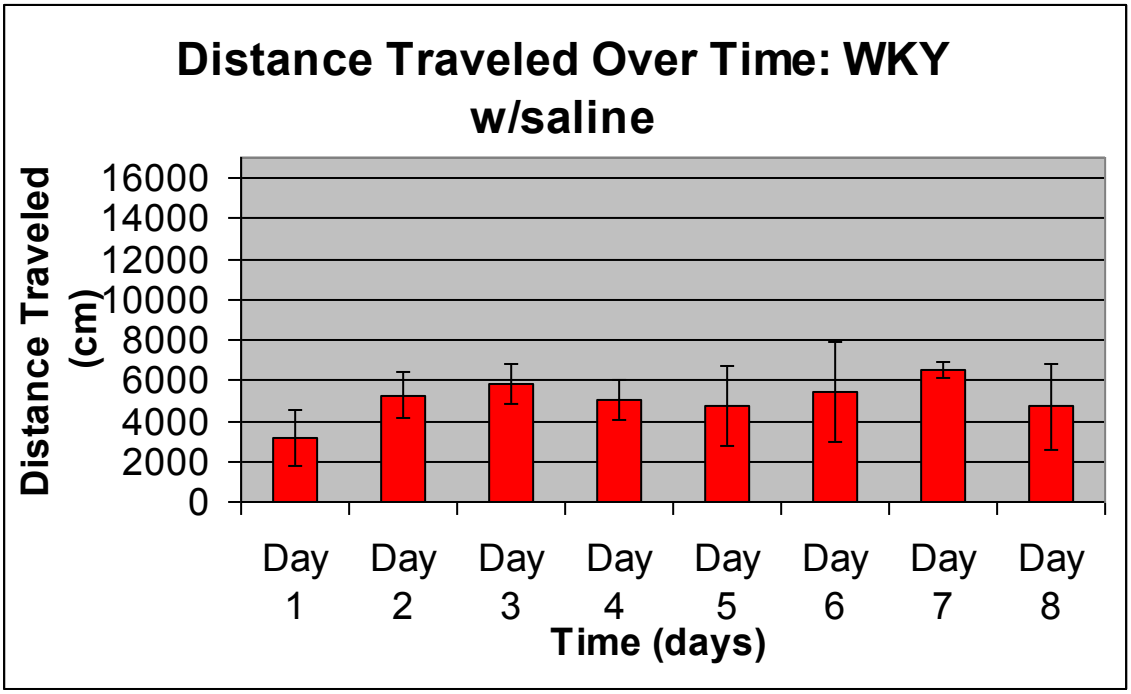

Figure 7: This figure shows the movement for an n=4 of WKY animals being injected with only saline. The movement stays basically constant at an average distance of 5000 cm.

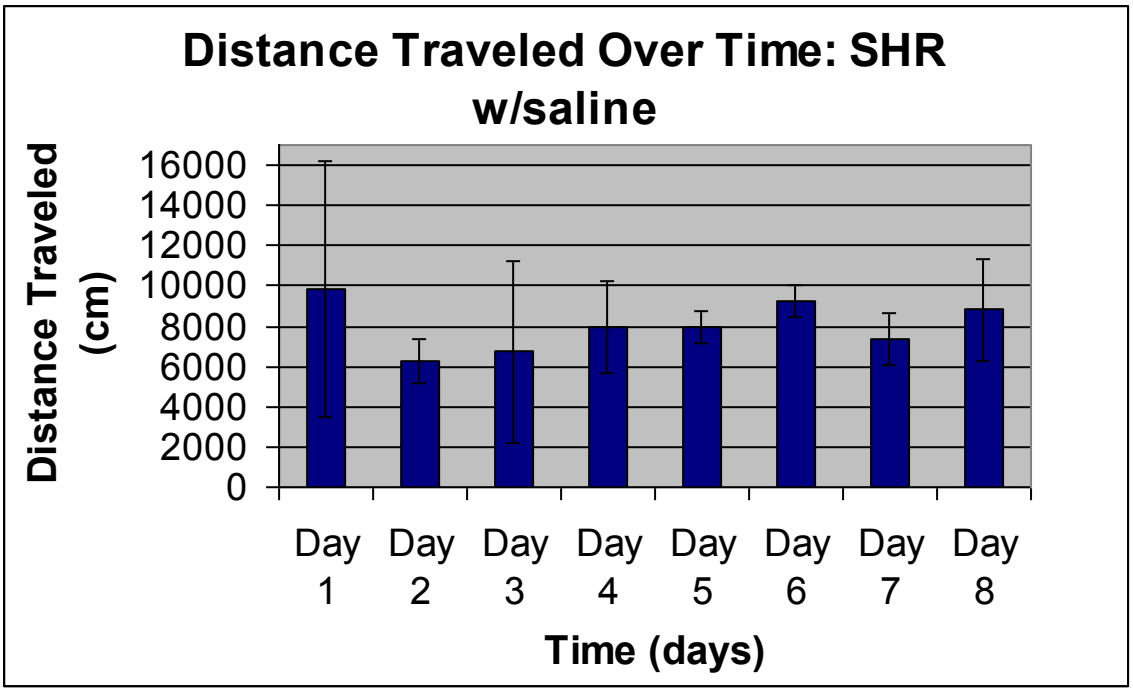

Figure 8: This figure shows the movement for an n=4 of SHR animals being injected with only saline. The movement stays basically constant at an average distance of 8000 cm.

The nicotine injections begin on Day 3, with the first two days as habituation.

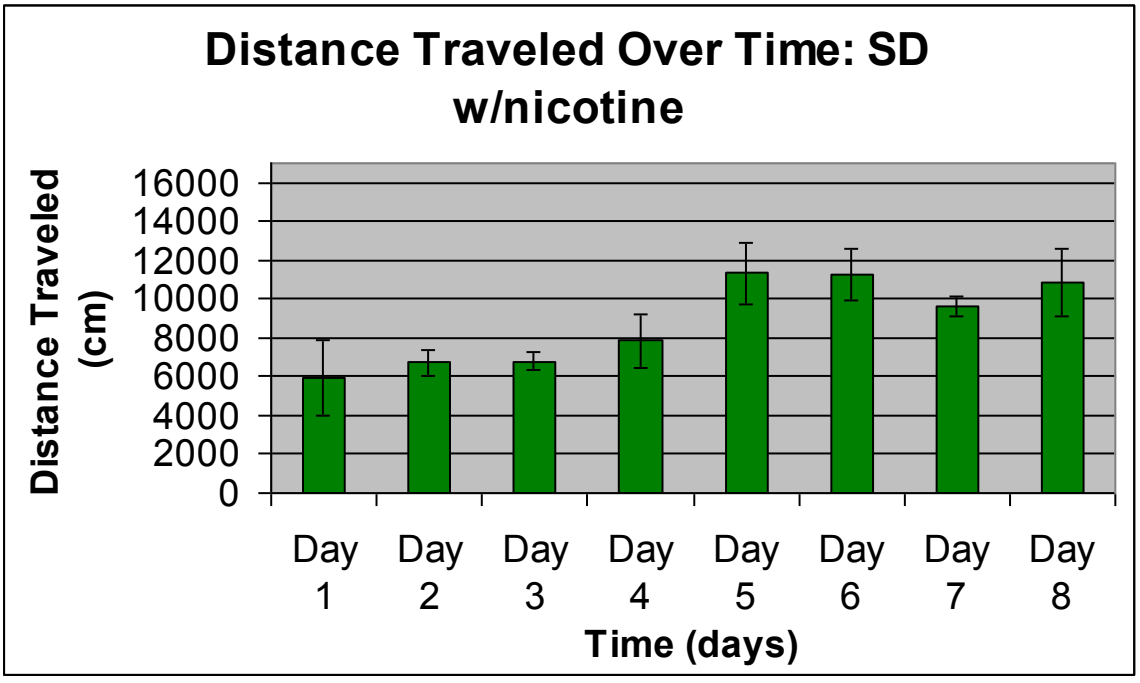

Figure 9: This figure shows the movement for an n=4 of SD animals being injected with nicotine. The movement increases as the daily injections are given. The animal is sensitized by Day 6, when the movement stops increasing and around 11000 cm.

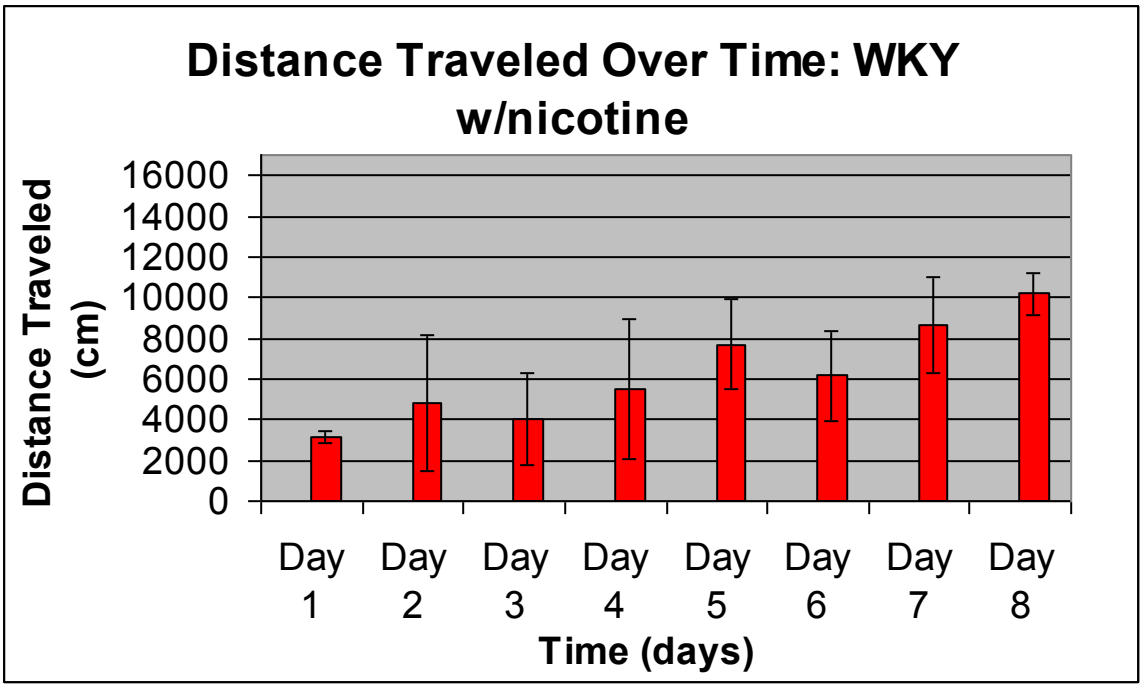

Figure 10: This figure shows the movement for an n=4 of WKY animals being injected with nicotine. The movement increases as the daily injections are given. The animal is sensitized by Day 8, when the movement stops increasing at around 10000 cm.

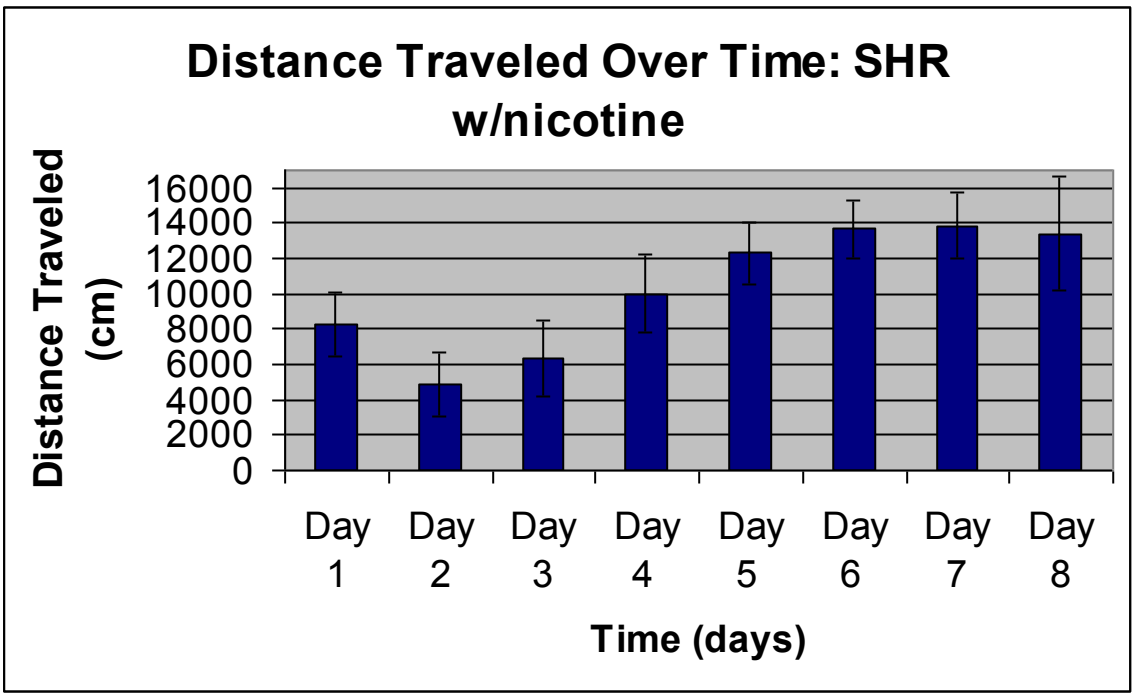

Figure 11: This figure shows the movement for an n=4 of SHR animals being injected with nicotine. The movement increases as the daily injections are given. . The animal is sensitized by Day 7 when the movement stops increasing at around 14000 cm.

The nicotine injections and the stress are both introduced on the third day, with the first two days as habituation.

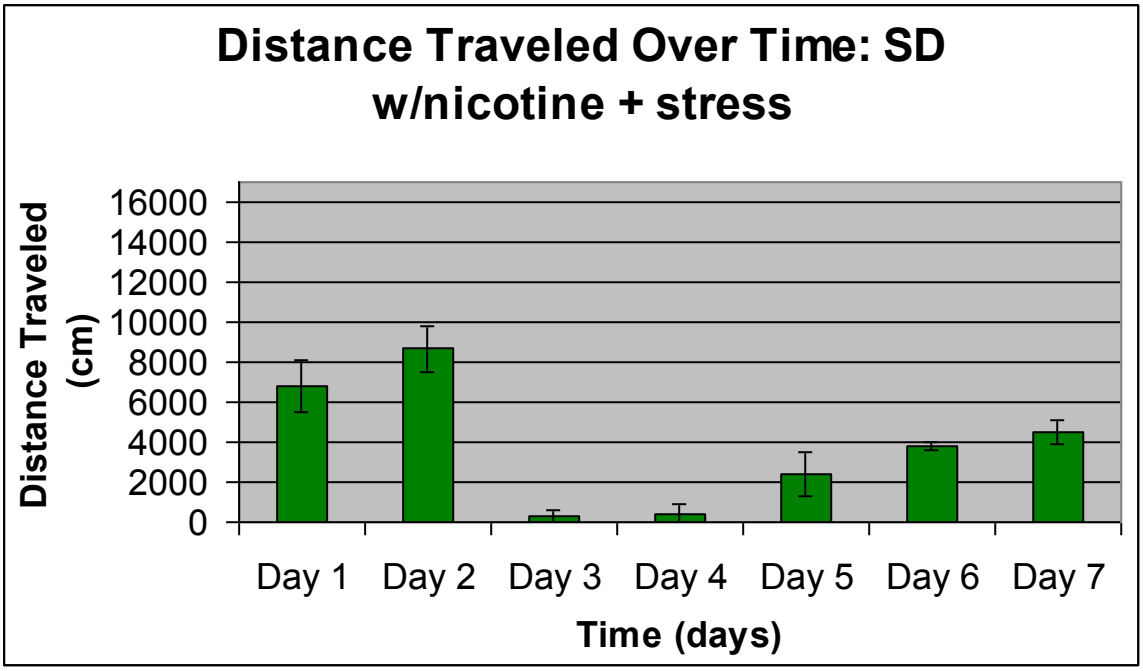

Figure 12: This figure shows the movement for an n=4 of SD animals being injected with nicotine with an added stress. The movement increases as the daily injections are given. . The animal is sensitized by Day 6 when the movement stops increasing at around 4500 cm.

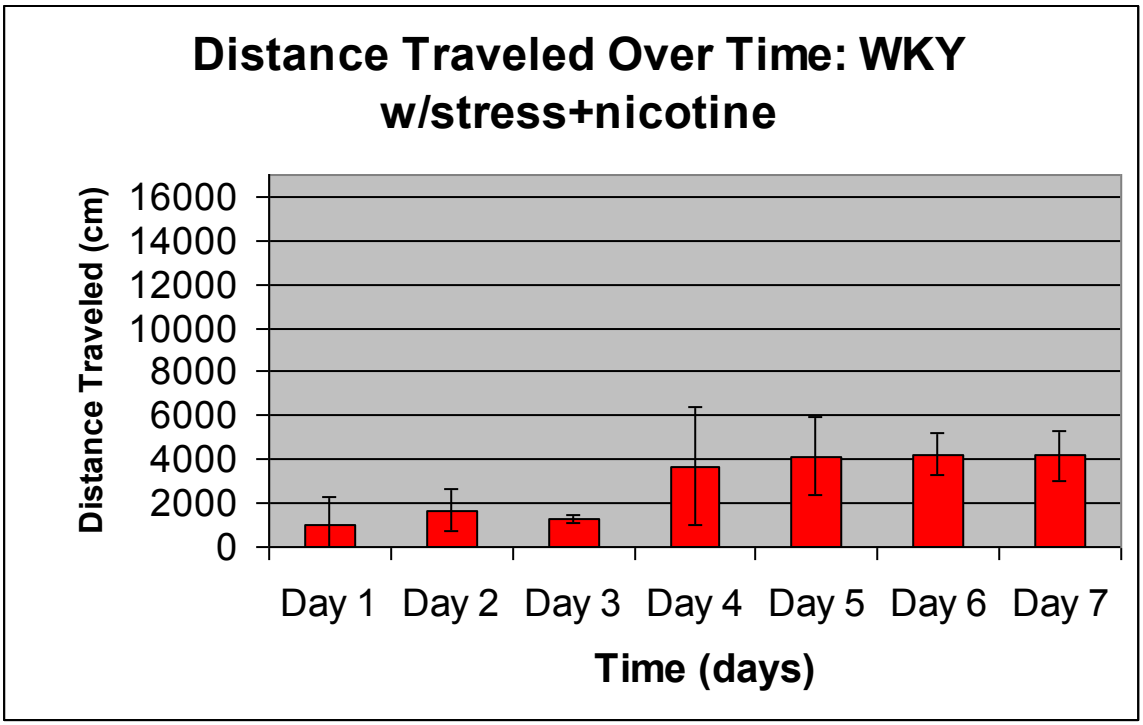

Figure 13: This figure shows the movement for an n=4 of WKY animals being injected with nicotine with an added stress. The movement increases as the daily injections are given. . The animal is sensitized by Day 5 when the movement stops increasing at around 4000 cm.

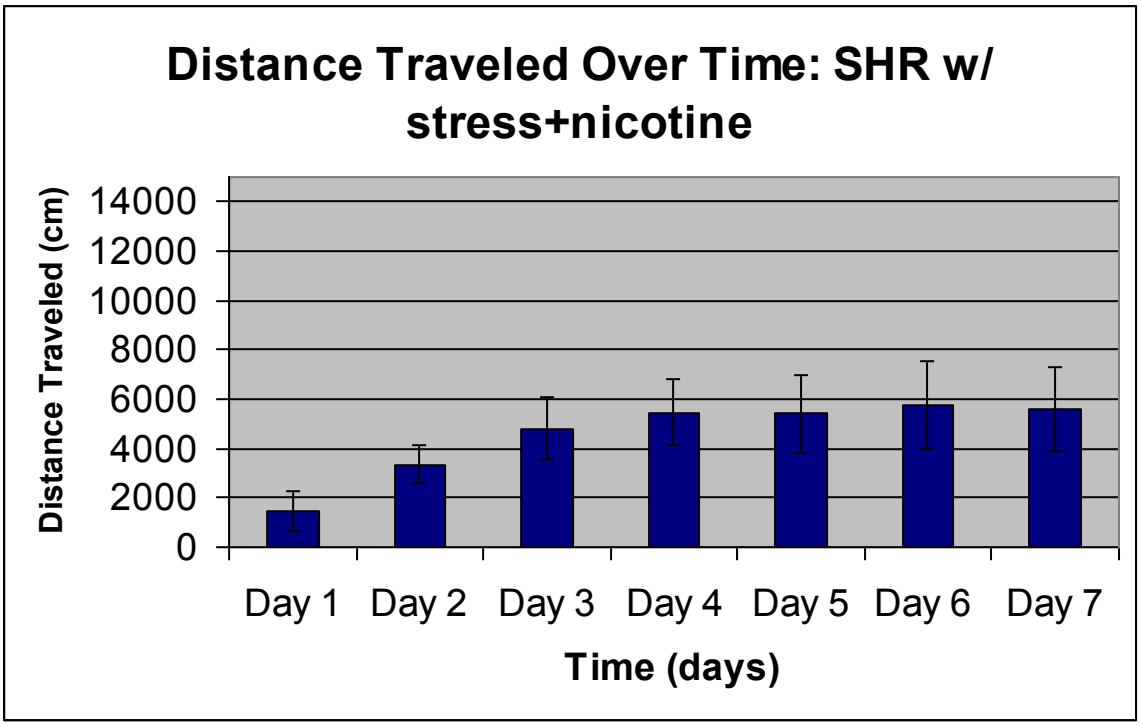

Figure 14: This figure shows the movement for an n=4 of SHR animals being injected with nicotine. The movement increases as the daily injections are given. . The animal is sensitized by Day 4, when the movement stops increasing at around 6000 cm.

Figures 12-14 show a leveling off of locomotor activity more rapidly and are contrary of what was predicted in the hypothesis.

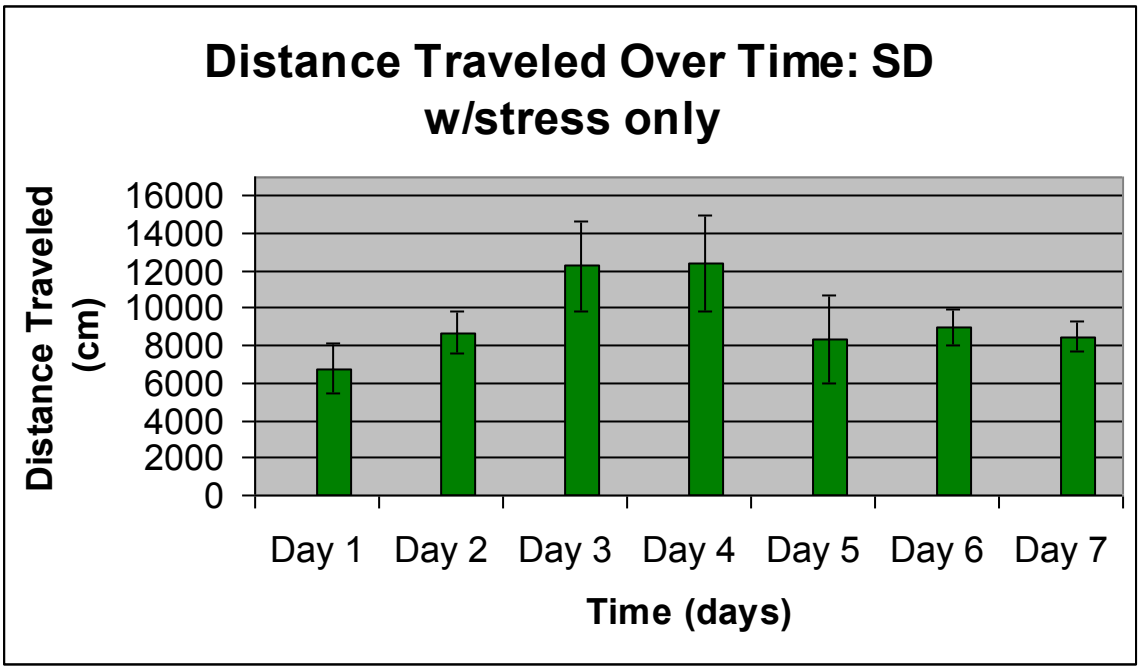

Figure 15: This figure shows the movement for an n=4 of SD animals being applied only a stress. The movement increases, and then appears to normalize at around 8000 cm.

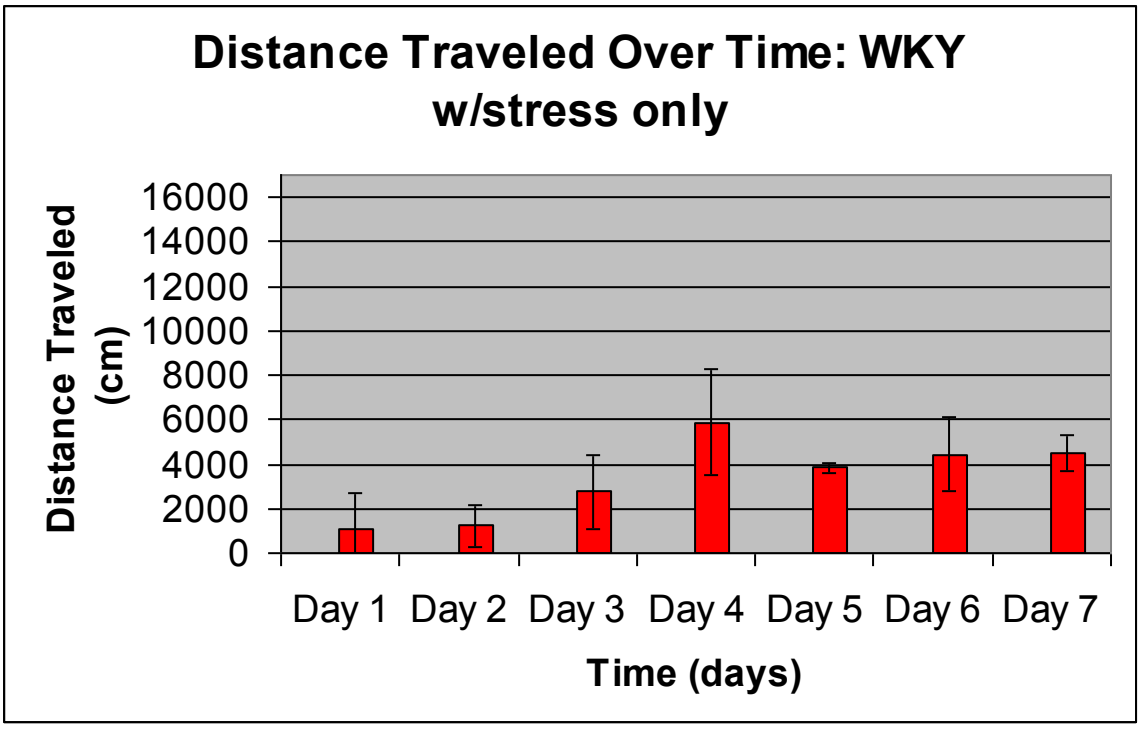

Figure 16: This figure shows the movement for an n=4 of WKY animals being applied only a stress. The movement increases, and then appears to normalize at around 4000 cm.

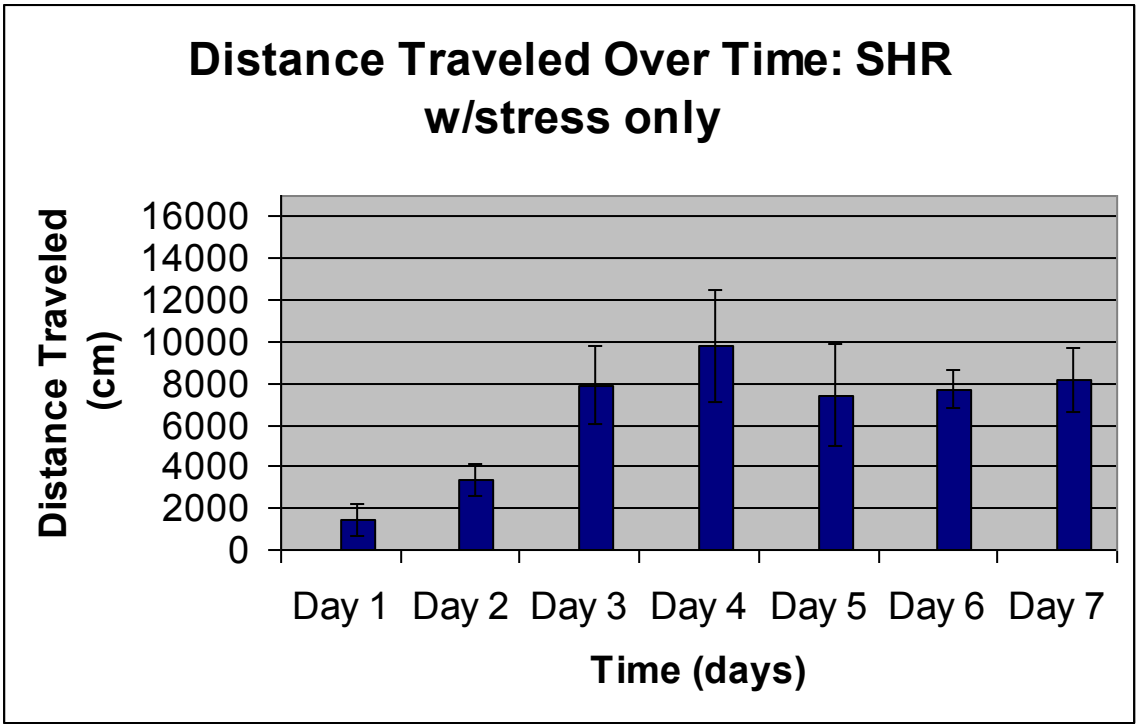

Figure 17: This figure shows the movement for an n=4 of WKY animals being applied only a stress. The movement increases, and then appears to normalize at around 8000 cm.

fMRI

The functional magnetic resonance imaging was conducted for an acute dosage of nicotine and also for nicotine sensitized animals of both WKY and SHR (both cases). Below presented is an image for each group of animals showing the activity in the reward system regions of the brain. The reward system of the brain contains the areas that are typically activated when a drug is given and motivates people to seek the substances that give pleasure and avoid substances that give physical discomfort, depression or social isolation. [33]

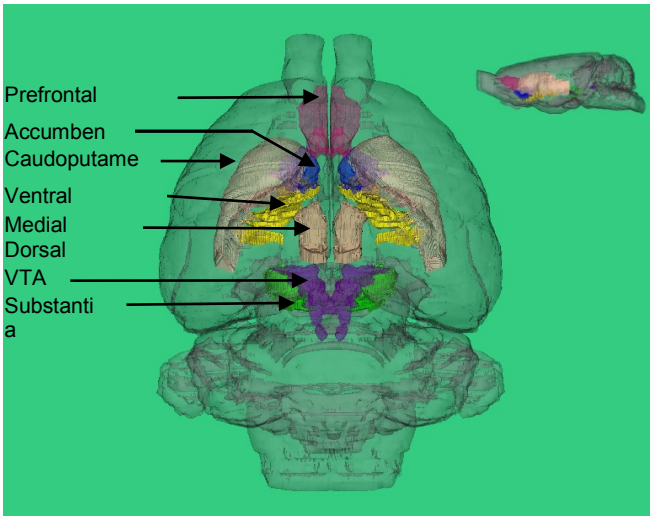

Figure 18: This figure shows the parts of the reward system of the brain, which is normally activated when a drug is given.

Out of the areas activated, the ventral tegmental area (VTA), the accumbens, and the prefrontal cortex play the most crucial roles in this system. For nicotine specifically, the areas most affected are the hippocampus and the cortex, which explains the increase in awareness and attentiveness that smokers report they feel.[34]

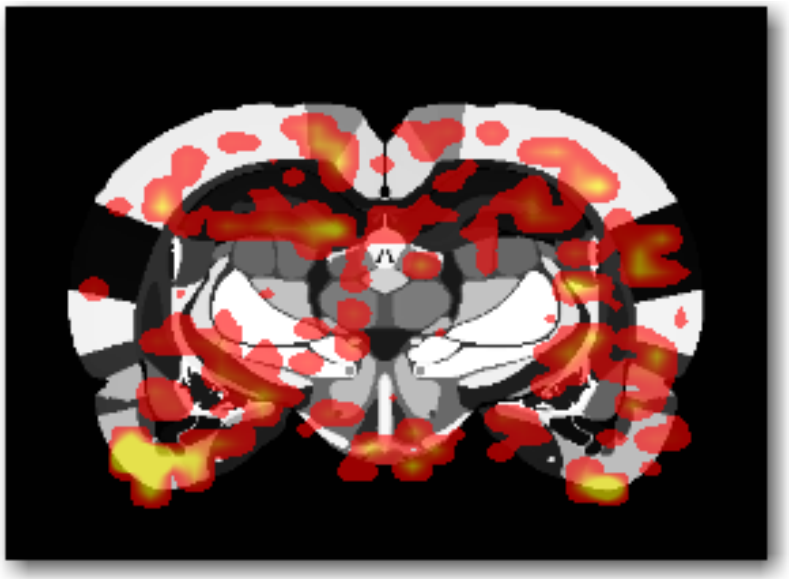

Figure 19: The figure above shows the activation in the WKY rat, the control, in the reward system of the brain for an acute nicotine dosage.

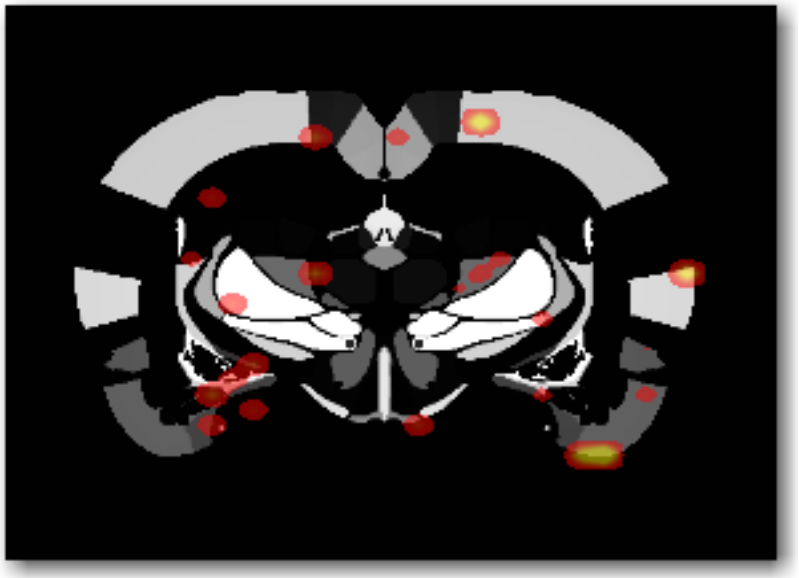

Figure 20: The figure above shows the activation in the SHR rat, the ADHD model, in the reward system of the brain for an acute nicotine dosage.

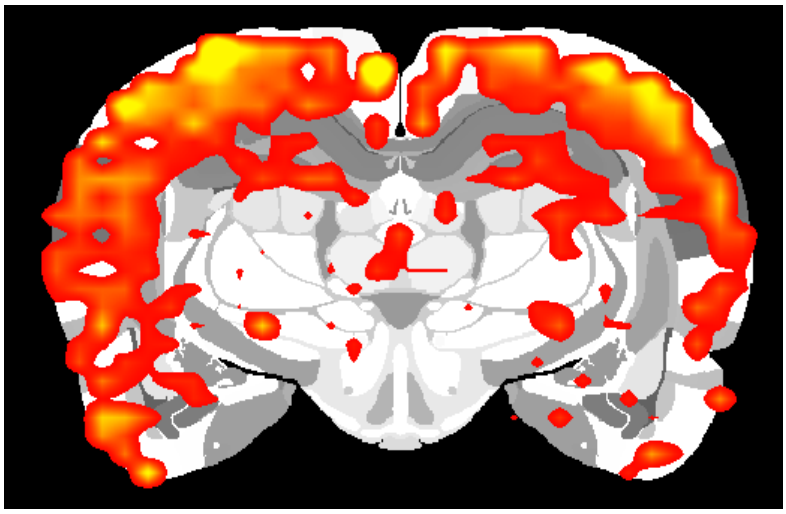

Figure 21: The figure above shows the activation in the WKY rat, the control, in the reward system of the brain once it has been sensitized. [35]

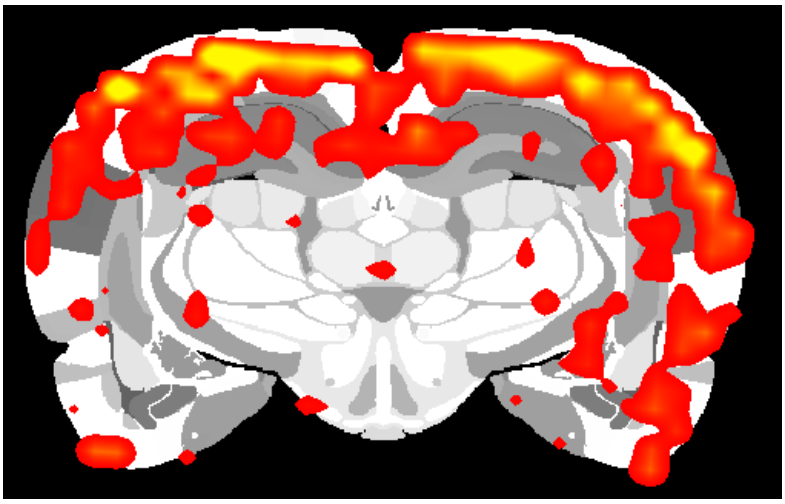

Figure 22: The figure above shows the activation in the SHR rat, the ADHD model, in the reward system of the brain once it has been sensitized. [36]

Data Analysis

Sensitization

From the above figures, it is shown that as a baseline, the movement for the SD rat stays basically constant at an average distance of 8500 cm. The movement for the WKY stays basically constant at an average distance of 5000 cm, and the movement for SHR at 8000 cm. The movement increases as the daily injections of nicotine are given and the SD animal is sensitized by day 6, when the movement stops increasing at about 11000 cm. The WKY animals are sensitized by Day 8, when the movement stops increasing at

around 10000 cm and the SHR animals are sensitized by Day 7 when the movement stops increasing at around 14000 cm. Finally, with the added stress, the movement once again increases as the daily injections of nicotine are given, however, at a faster rate. The SD animals are sensitized by Day 6, when the movement stops increasing at about 4000 cm. The WKY animals are sensitized by Day 5, when the movement stops increasing at around 4000 cm and the SHR animals are sensitized by Day 4 when the movement stops increasing at around 6000 cm. This data is summarized below, and it is easy to see the differences in locomotion between the rats in each circumstance.

- Saline
  - WKY: 5,000 cm.
  - SHR: 8,000 cm.
  - SD: 8,500 cm.
- Nicotine
  - WKY: 10,000 cm.
  - SHR: 14,000 cm.
  - SD: 11,000 cm.
- Nicotine + Stress
  - WKY: 4,000 cm.
  - SHR: 6,000 cm.
  - SD: 4,500 cm.

The difference in intensity of sensitization between the WKY rat, which is considered to be more the control, and the SHR rat, the ADHD model, can be better seen with a percent change graph. Although both animals have approximately the same change by day 4, the SHR rat reaches this point much faster.

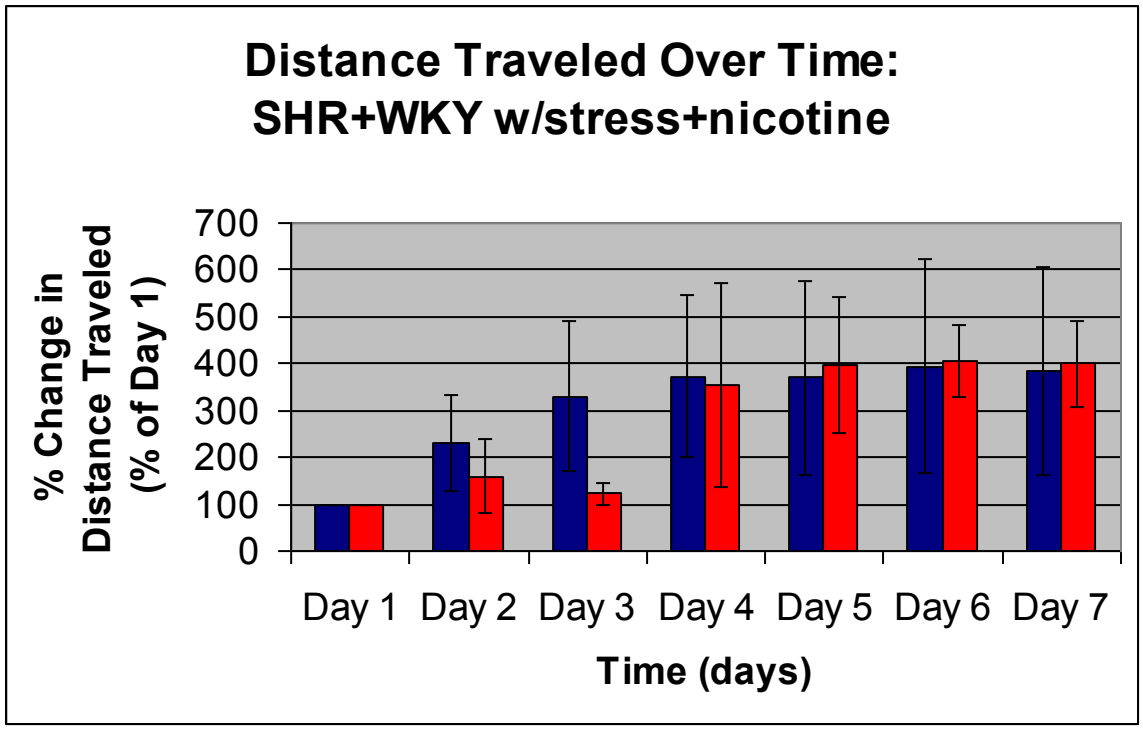

Figure 23: This figure shows the % change in movement comparing WKY in red to SHR in blue.

MRI

The fMRI images shown above can be graphically represented, showing the percentage of activity in each specific brain region of the reward system.

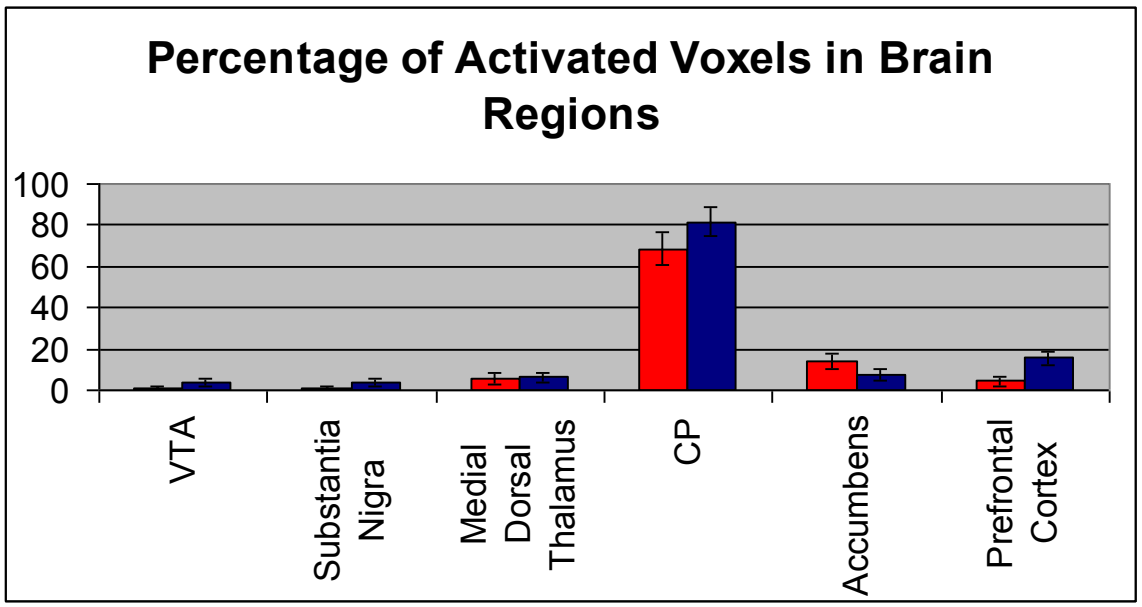

Figure 24: This figure shows the % of activated voxels in each of the brain areas listed in the reward system regions in the control (red) and the ADHD model rat (blue).

As can be seen, there is very little to no difference in the percentage of activity in these regions, although the SHR has greater activity. However, from the images gathered, there is a clear difference in brain activity between the WKY and the SHR. Looking specifically at nicotine, it was already mentioned that the cortex is one of the areas most affected. This is confirmed by the graph below.

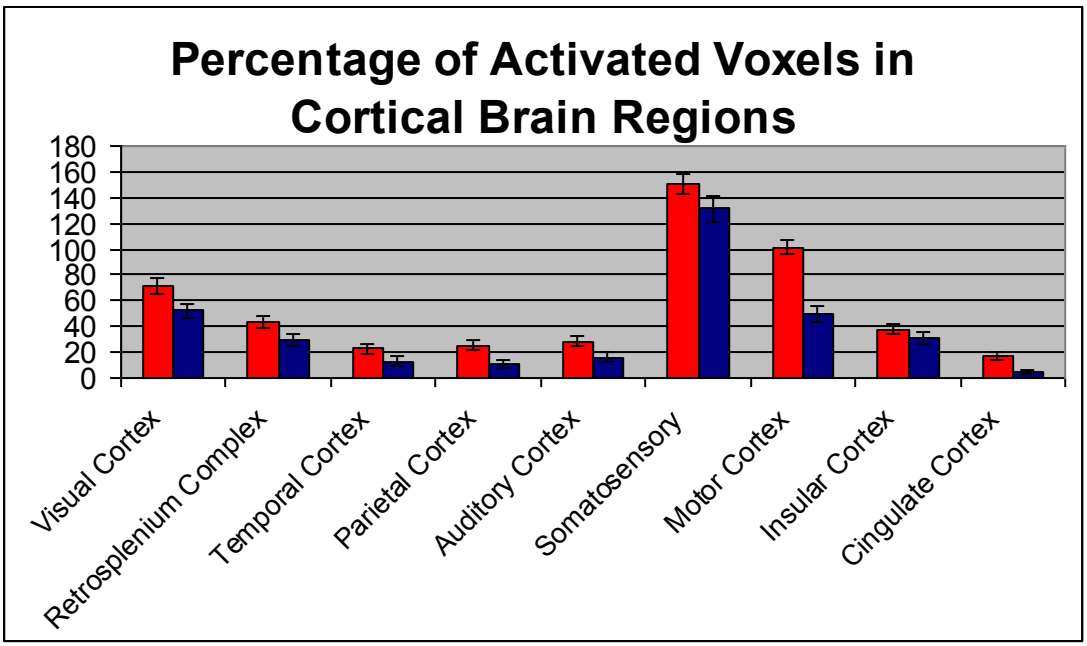

Figure 25: This figure shows the % of activated voxels in each of the brain areas listed cortical regions (senses) in the control (red) and the ADHD model rat (blue).

Both models the experience the nicotine as a reward, so there is no difference brain activation in the reward system. However, the ADHD model shows decrease in activation in cortical regions.

Conclusion

Behaviorally, movement increased for both the ADHD and control models when administered nicotine, and when stress was added, the movement for both groups was reduced to below the baseline average values. A more rapid sensitization was measured in both animal models with stress. The ADHD model, SHR rat, sensitized to nicotine more quickly than the control under the stress and non-stress conditions. For the acute nicotine dose that was given during the fMRI, the ADHD model showed less activation in the brain than the control. The data showed a greater nicotine sensitization for the ADHD model compared to the non-ADHD model, the control. However, sensitization was not amplified when stress was coupled to nicotine for both models, contrary to the hypothesis. The fMRI results corroborate these findings. This study was intended to be a pilot study, with the fMRI data showing that there are corresponding neurological changes paired with the behavioral data.

References

[1] Biederman, J. Attention-Deficit/Hyperactivity Disorder: A Selective Overview. Biol Psychiatry 2005: 57:1215-1220.
[2] Biederman, J. Attention-Deficit/Hyperactivity Disorder: A Selective Overview. Biol Psychiatry 2005: 57:1215-1220.
[3] Biederman, J. Attention-Deficit/Hyperactivity Disorder: A Selective Overview. Biol Psychiatry 2005: 57:1215-1220.
[4] Kreek MJ, Nielsen DA, Butelman ER, LaForge KS. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nature Neurosci 2005:8:1450-1458.
[5] Medical Network Inc."Stress." Health A to Z: Your e_Health Solution. 26 April 2006. 1999-2006 <www.healthatoz.com>
[6] Al'Absi M. Hypothalamin-pituutary-adrenocortical responses to psychological stress and risk for smoking relapse. Int J Psychophysiol 2006:59(3):218-27
[7] Al'Absi M. Hypothalamic-pituutary-adrenocortical responses to psychological stress and risk for smoking relapse. Int J Psychophysiol 2006:59(3):218-27
[8] Al'Absi M. Hypothalamic-pituutary-adrenocortical responses to psychological stress and risk for smoking relapse. Int J Psychophysiol 2006:59(3):218-27
[9] Al'Absi M. Hypothalamic-pituutary-adrenocortical responses to psychological stress and risk for smoking relapse. Int J Psychophysiol 2006:59(3):218-27
[10] Lovallo WR. Cortisol secretion patterns in addiction and addiction risk. Int J Psychophysiol 2006:59(3):195-202
[11] Lovallo WR. Cortisol secretion patterns in addiction and addiction risk. Int J Psychophysiol 2006:59(3):195-202
[12] Lovallo WR. Cortisol secretion patterns in addiction and addiction risk. Int J Psychophysiol 2006:59(3):195-202
[13] Williams JM, Ziedonis D. Addressing tobacco among individuals with a mental illness or an addiction. Addict Behav 2004:29:1067-1083.
[14] Saito M, O'Brien D, Kovacs KM, Wang R, Zavadil J, Vadasz C. Nicotine-Induced Sensitization in Mice: Changes in Locomotor Activity and Mesencephalic Gene Expression. Neurochem Res 2005:30(8):1027-1035
[15] Saito M, O'Brien D, Kovacs KM, Wang R, Zavadil J, Vadasz C. Nicotine-Induced Sensitization in Mice: Changes in Locomotor Activity and Mesencephalic Gene Expression. Neurochem Res 2005:30(8):1027-1035
[16] Saito M, O'Brien D, Kovacs KM, Wang R, Zavadil J, Vadasz C. Nicotine-Induced Sensitization in Mice: Changes in Locomotor Activity and Mesencephalic Gene Expression. Neurochem Res 2005:30(8):1027-1035
[17] Hitzemann R. Animal Models of Psychiatric Disorders and Their Relevance to Alcoholism. Alcohol Health Res World 2000:24(3):149-176
[18] Russell VA, Sagvolden T, Johansen EB. Animal models of attention-deficit hyperactivity disorder. Behav Brain Funct:2005:15:1-9
[19] Sagvolden T, Johansen EB, Aase H, Russel VA. A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. Behav Brain Sci: 2005:28(3):397-419
[20] Russell VA, Sagvolden T, Johansen EB. Animal models of attention-deficit hyperactivity disorder. Behav Brain Funct:2005:15:1-9
[21] Sagvolden T, Russel VA, Aase H, Johansen EB, Farshbaf M. Rodent models of attention-deficit/hyperactivity disorder. Biol Psychiatry:2005:57(11):1239-1247
[22] Sagvolden T, Russel VA, Aase H, Johansen EB, Farshbaf M. Rodent models of attention-deficit/hyperactivity disorder. Biol Psychiatry:2005:57(11):1239-1247
[23] Febo M, Numan M, Ferris CF. Functional Magnetic Resonance Imaging Shows Oxytocin Activates Brain Regions Associated with Mother-Pup Bonding druing Suckling. J Neurosci:2005:25:11637-11644
[24] Hornak, James. The Basics of MRI. 2006. < http://www.cis.rit.edu/htbooks/mri/>

[25] King JA, Garelick TS, Brevard ME et al. Procedure for minimizing stress for fMRI studies in conscious rats. J Neurosci Methods 2005: 148:154-160
[26] Lexico Publishing Group, LLC. "Habituation." 2006. <www.dictionary.com>
[27] Saito M, O'Brien D, Kovacs KM, Wang R, Zavadil J, Vadasz C. Nicotine-Induced Sensitization in Mice: Changes in Locomotor Activity and Mesencephalic Gene Expression. Neurochem Res 2005:30(8):1027-1035
[28] Kosowski AR, Liljequist S. Behavioural sensitization to nicotine precedes the onset of nicotine-conditioned locomotor stimulation. Behav Brain Res 2005:156:11-17.
[29] McCormick CM, Robarts D, Gleason E, Kelsey JE. Stress during adolescence enhances locomotor sensitization to nicotine in adulthood in female, but not male, rats. Horm Behav 2004:46:458-466
[30] Cadoni, 2000; Collins and Izenwasser, 2004; Collins and Montano, 2004; Kanyt, 1999 etc.
[31] University of Florida. "Animal Care Services. 2006.
<http://acs.ufl.edu/guidelines/bloodCollection.shtml>
[32] McDougall SJ, Widdop, RE, Lawrence AJ. Differential gene expression in WKY and SHR brain following acute and chronic air-puff stress. Molecular Brain Research 2005: 133:329-336.
[33] The Canadian Institutes of Health Research. "The brain from top to bottom." 2002-present.
<http://www.thebrain.mcgill.ca/flash/i/i_03/i_03_cr/i_03_cr_par/i_03_cr_par.html>
[34] The Canadian Institutes of Health Research. "The brain from top to bottom." 2002-present.
<http://www.thebrain.mcgill.ca/flash/i/i_03/i_03_cr/i_03_cr_par/i_03_cr_par.html>
[35] Kulkarni, P. Personal Correspondence. 2006.
[36] Kulkarni, P. Personal Correspondence. 2006.