EFFECTS OF CHEMICAL COMPOUNDS FOUND IN CIGARETTE SMOKE ON COGNITIVE ABILITY OF MICE

by

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ABSTRACT

The purpose of this experiment was to find a conclusive link between smoking and its effects on the development of Alzheimer's disease. This was evaluated by determining whether certain elements of cigarette smoke affect cognitive ability of mice. Five different groups of mice (control, ammonium hydroxide exposure, methanol exposure, acrylamide exposure, cigarette smoke exposure) attempted to navigate a water maze to complete the Morris water escape task. The experimental method was as follows: the five groups of mice were exposed to the suspected amyloid-inducing chemicals dissolved in their drinking water. The chemical concentrations were proportional to the concentrations human smokers are exposed to in Marlboro Red Full Flavor cigarettes. Over a three week duration, the mice completed the Morris water escape task seven times, with their time to completion recorded. The expected trend was that exposure to chemicals found in cigarette smoke would lead to an increase in the time it takes for that group of mice to navigate the maze. The results obtained actually contradicted this hypothesis: it was found that the control group had the fourth best time amongst the five groups, and one of the experimental groups (acrylamide exposure) was significantly better at solving the maze than the rest. However, these results can only be regarded as preliminary; further testing must be conducted to more positively determine the correlation between chemical exposure and mouse cognitive ability.

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CHAPTER ONE: INTRODUCTION

In the 21st century, the fact that cigarette smoking increases the risk of various types of cancers is a well-known and widely accepted fact. However, with the sheer number of chemicals present in the average cigarette – approximately 600, and over 4,000 upon combustion – researchers have questioned whether cigarette smoking can lead to an assortment of non-cancer-related diseases (www.tobacco.org). Recent studies have pointed toward the fact that smoking leads to the development of certain dementias such as Alzheimer's Disease (AD), and vascular dementia. Being the most common form of dementia, AD has received the most attention thus far. These diseases are characterized by a loss of memory and commonly affect the elderly. Patients with AD in particular exhibit unique developments in the brain. The first of these is the presence of abnormal tangles of tau proteins in the brain. The second is the presence of beta-amyloid (βA) plaques which form deposits in the brain. Support for the presence of βA plaques is derived from the location of amyloid precursor protein on chromosome 21. Chromosome 21 trisomies result in Down Syndrome, and patients suffering from Down Syndrome almost always exhibit AD by age 40. Tau proteins are believed to cause neurofibrillary tangles inside nerve cell bodies by pairing with other hyperphosphorylated tau proteins. This leads to disintegration of microtubules, which are critical structural components of the neuron. The result of this is dysfunction in biochemical communication between neurons. A primary compound that is being labeled a cause for the development of dementia is exposure to type-2 alkenes, which are commonly present in cigarette smoke (Weinhold, 2011). Experiments to date have generally

noted an increase in the risk for both AD and vascular dementia. However, conflicting data has been presented that smoking helps remedy the effects of AD.

In order to address the question posed, we designed an experimental method to be able to determine whether there is a conclusive relationship between the chemicals in cigarette smoke and the onset of dementia. The purpose of the experiment was to determine which chemicals (if any) have an effect on the onset of dementia. Laboratory mice were used as model organisms, with a decline in cognitive function being equated with AD-like symptoms. But in actuality, it proved rather difficult to pinpoint how exactly dementia has set in for numerous reasons. The first of these is that dementia generally takes a while to show up in human beings. Dementia is usually manifested in late adulthood, around the late-sixties to early-seventies. Therefore, it is possible that dementia takes a long time to set in, or that it does not occur until late in a person's life. In the first instance, the assumption is that the cause of dementia is smoking in middle age (around late-forties) but the effects of the smoking (i.e. dementia) are not manifested until almost three decades later. In the second scenario, it is potentially possible that dementia does not occur readily in anybody not in late adulthood. It proved difficult to replicate either of these scenarios because mice were utilized in the experiment. Mice have a different developmental time frame than do humans; therefore, it is necessary to account for this in order to make inferences regarding the data from the mice that apply to humans. Therefore, for the aforementioned reasons we believe our experiment will provide more useful data to us if we examine the effect that cigarette chemicals have on the cognitive function of mice, particularly their memory and ability to learn.

CHAPTER TWO: BACKGROUND

Many articles have been compiled that analyze the connection between smoking and the onset of dementia. Researchers have generally concluded that smoking does play a definitive role in the development of AD and vascular dementia. However, it has not yet been determined precisely what chemicals or ingredients in cigarettes actually affect the presence of dementia in an individual. Yet on the other hand, a few articles have actually claimed the opposite: that smoking may help to prevent the development of dementia. These reports claim that some of the compounds in the average cigarette, particularly nicotine, actually combat the proliferation of plaques as AD first begins to surface in an individual. But it is important to note that most of these reports were funded by groups with vested interests in the tobacco industry, and therefore the information may likely be biased (Leong, 2010).

In one study, researchers at Kaiser Permanente in Oakland, California, and at the University of Eastern Finland in Kuopio conducted a longitudinal study to determine the effect that smoking had on the average elderly person's health. Approximately 21,000 middle-aged patients (around 50 years old) were selected. Their smoking histories were analyzed then they were followed for the next 20 years. By the time the study was completed, the subjects were an average of 71.6 years, which is right within the age range that AD begins to manifest itself. Their conclusion was that people who smoked were at much higher risk for developing dementia: those who smoked two packs a day during middle age were over 150% more likely to develop AD and over 170% likely to develop vascular dementia. But they also noted that those who smoked prior to middle age and then quit, or those who smoked less than half a pack a day during middle age

did not seem at greater risk for developing dementia. This appears to indicate that the harmful effects of smoking on dementia onset do not last from youth, but are only induced in late middle age as dementia begins to develop. In addition, they were able to rule out both gender and ethnicity as factors that would influence the rate of dementia within a particular demographic (Anstey et al., 2007).

Another study followed a group of smokers in middle age but actually tested their cognitive function to determine the magnitude of damage to their brains. About 26,000 subjects were followed for 2-30 years with another 17,000 followed for 2-7 years, with the mean age being 74 years. Current smokers were determined to be approximately 1.8 times more likely to develop AD and vascular dementia than those who had never smoked. In a similar fashion, the smokers exhibited greater declines in the Mini-Mental State Examination that was administered to them annually. Therefore, they concluded that elderly people who smoked were at much greater risk for acquiring dementias and experiencing cognitive decline (Barth, 1997).

On the other side of the debate, two studies in particular have reported that smoking promotes the delay of AD onset. The first study was an analysis of present data regarding smoking and dementia. About a quarter of the data analyzed was compiled by researchers with ties to the tobacco industry. Based on these reports, the statisticians concluded that smoking protects against AD. But when working with the numbers from non-tobacco related studies, the researchers found that there was indeed a significant risk for developing AD when a person smokes during middle age (Cataldo et al., 2010). Another study revealed that nicotine may help to reverse the signs of AD. Nicotine was administered to patients intravenously and a decrease

in intrusion errors was noted with a certain dosage. They hypothesized that nicotine plays a role in cholinergic stimulation and thus may be a potential treatment for AD (Newhouse et al., 1988).

Two other studies led to the discovery of important pieces of information that may help us to better understand the relationship smoking has with dementia. The China-based Third Military Medical University conducted an experiment where they injected amyloid plaques into the brains of healthy rats, then gave some an equivalent dose of nicotine for two weeks. Both groups of rats displayed the presence of tau tangles and did poorly when navigating a maze, but the rats that received nicotine did markedly worse than the rats that received none (Zukerman, 2010). The second major discovery is that of type-2 alkenes. These chemicals have been found to be widely abundant in the environment; their presence runs the gamut from cigarette smoke to car exhaust fumes to French fries. Researchers have shown that these chemicals damage the nerve endings in the brains of animals, and that the type-2 alkenes have been found to be present in the brains of those with dementia (Rettner, 2010).

One journal article in particular highlighted the effects of the type-2 alkene, acrolein. Bob Weinhold of the Society of Environmental Journalists explained that the chemical is one of the leading culprits of neurologic disorders. Acrolein is actually produced naturally in the body as a by-product of membrane lipid oxidation. It is also produced as a by-product in combustion reactions, which is why it is found in car exhaust fumes and cigarette smoke. The EPA already knows that it damages respiratory function, but not much is known regarding its effects on the brain. A recent study by Gary Leung of Purdue University may prove that acrolein does indeed induce neurological disorders (Leung et al., 2011). Mice were injected with a substance known

to cause experimental autoimmune encephalomyelitis, which is essentially a form of multiple sclerosis for mice. After approximately two weeks, the levels of acrolein in the spinal cord increased by about 65%. At the same time, the mice began to exhibit severe muscle control problems. Because of this, Leung believes that environmental acrolein could provide the same amount of damage that the naturally-produced acrolein does. And the fact that acrolein is just one of many type-2 alkenes – including acrylamide, methyl vinyl ketone, and 4-hydroxy-2-nonenal (HNE) – means that the potential for neurological damage is much greater than was anticipated (Weinhold, 2011).

In recent years, researchers have reported on the role that synaptic dysfunction plays in the progression of certain neurodegenerative diseases such as AD, amyotrophic lateral sclerosis (Lou Gehrig's disease), and Parkinson's disease. It has been speculated that these diseases share a common pathophysiological cascade that includes oxidative stress, the peroxidation of lipids, and liberation of type-2 alkenes such as acrolein and HNE. *In vivo* and *in vitro* data have revealed that such electrophilic chemicals cause extensive damage at nerve terminals via the formation of Michael-type adducts with nucleophilic sulfhydryl groups on presynaptic proteins. The effects of type-2 alkenes on brain synaptotoxicity were studied by Lopachin et al. Their results provide evidence that damage to the synapses may occur in localized regions of the brain, and that this process is mediated by the generation of endogenous acrolein and HNE. In addition, the acceleration of synaptotoxicity may occur via exposure to environmental type-2 alkenes (Lopachin et al., 2008 (b)). In another experiment by Lopachin et al., the potency for synaptotoxicity of another type-2 alkene, acrylamide, was analyzed. This study attempted to unearth more information about the molecular mechanism that was responsible for the

toxification of brain synapses. The relationships among a series of conjugated type-2 alkenes were studied via exposure to rat striatal synaptosomes. It was found that these chemicals produced concentration-dependent neurotoxic effects that correlated to the loss of free sulfhydryl groups. These results may indicate that the mechanism of Michael adduction on α,β-unsaturated carbonyls may play a major role in the onset of neurogenerative diseases such as Alzheimer's (Lopachin et al., 2007).

The efficacy of the type-2 alkene acrylamide on neurodegeneration has been investigated more thoroughly within the past decade. Lopachin et. al claim that acrylamide (ACR) has clear neurotoxic effects in both animals and humans. Their results point to the conclusion that the nerve terminal is the primary site of ACR action and that inhibition of neurotransmission mediates the proliferation of neurological impairment. Specifically, the researchers have postulated that ACR inhibits neurotransmission by disrupting presynaptic nitric oxide (NO) signaling (Lopachin et al., 2008 (a)). Further testing allowed them to conclude that ACR can be considered a prototype of chemicals that induce central-peripheral distal axonopathy. These axonopathies are manifested by neurofilamentous swellings and eventual dengeneration of distal axon regions in both the CNS and PNS. Despite this, they determined that the hallmark lesion of ACR neurotoxicity is actually nerve terminal degeneration. Preliminary results indicate that ACR may also affect SNARE protein complexes (soluble NSF attachment protein receptors) via an adduction mechanism (Lopachin et al., 2002). SNARE proteins play a role in the assembly of fusion core complexes, and thus disruption to their function would certainly interfere with neurotransmission.

Previous studies of ACR neuropathy in the rat peripheral nervous system and cerebellum have suggested that axon degeneration was not a primary effect of the chemical. Thus, Lehning et al. conducted an experiment to morphologically examine ACR neurotoxicity in the rat central nervous system. Cupric silver stain was utilized to highlights characteristics of degeneration of various parts of nerve cells. Rats were exposed to ACR at two concentrations, 50 mg/kg per day or 21 mg/kg per day, and at different times the brains and spinals cords of rats were removed and stained. Results showed that regardless of dose rate, ACR intoxication produced early and progressive nerve terminal degeneration. Moreover, spatiotemporal analysis suggested that degeneration began at the nerve terminal then moved with time in the general direction of the nerve cell body (Lehning et al., 2002).

CHAPTER THREE: SIGNIFICANCE

The question, does smoking influence the development of dementia, is important because of the abnormally high correlation between people with dementia and a history of smoking. The correlation is high enough that it cannot be simply overlooked, but ought to be investigated to determine conclusively if cigarette smoking has anything to do with the presence of dementia. A big motivation for the research is the fact that the cause of AD is still considered unknown. In just a few cases (less than 5%) the cause has been linked to genetics, but apart from these rare instances experts in the health professions have been unable to readily identify any definitive causes or risk factors for the acquiring the condition. As previously stated, the link between the presence of dementia and a smoking lifestyle is prevalent enough for cigarettes to be investigated as a potential cause.

We believe that our methods will expand the research previously conducted in this particular area because thus far, no definitive experiments have been carried out to determine which ingredient(s) found in cigarette smoke actually cause the neurodegenerative effects, assuming that there is a positive correlation between cigarette smoking and dementia. In a few studies, scientists have administered nicotine to test animals and studied the effect it had on them. However, cigarettes contain almost 600 chemicals, each of which may play a role in the development of dementia. This possibility is only increased the more when one considers the near-infinite number of possible interactions that could happen between all these chemicals. In addition, it has been found that many of these substances are drastically altered in their chemical composition, structure, and function by combustion (i.e., burning), which is the most common

form of using tobacco products. We hope to zero in on a few other key chemicals found in cigarettes, particularly the type-2 alkenes, to determine if they have a definitive effect on the development of AD and other forms of dementia.

CHAPTER FOUR: MATERIALS

- CF-1 Strain Mice (30)
- 35 gallon steel Tub, 31.5-inch diameter
- Mouse feed
- Acrylamide, >99% (for gel electrophoresis)
- Ammonium Hydroxide, 32%
- Methanol, 90%
- Marlboro Red Full Flavor cigarettes

CHAPTER FIVE: METHODS

Overview

The memory function of the mice was assessed by subjecting several groups to the Morris water escape task. The task comprised placing the mice in a Morris water maze (see Fig. 1) and measuring the latency (the time taken to locate the platform) of each mouse. One group of mice served as a control, and other groups were exposed to various active ingredients of cigarettes for a total of five groups (control, ammonium hydroxide exposure, methanol exposure, acrylamide exposure, cigarette smoke exposure).

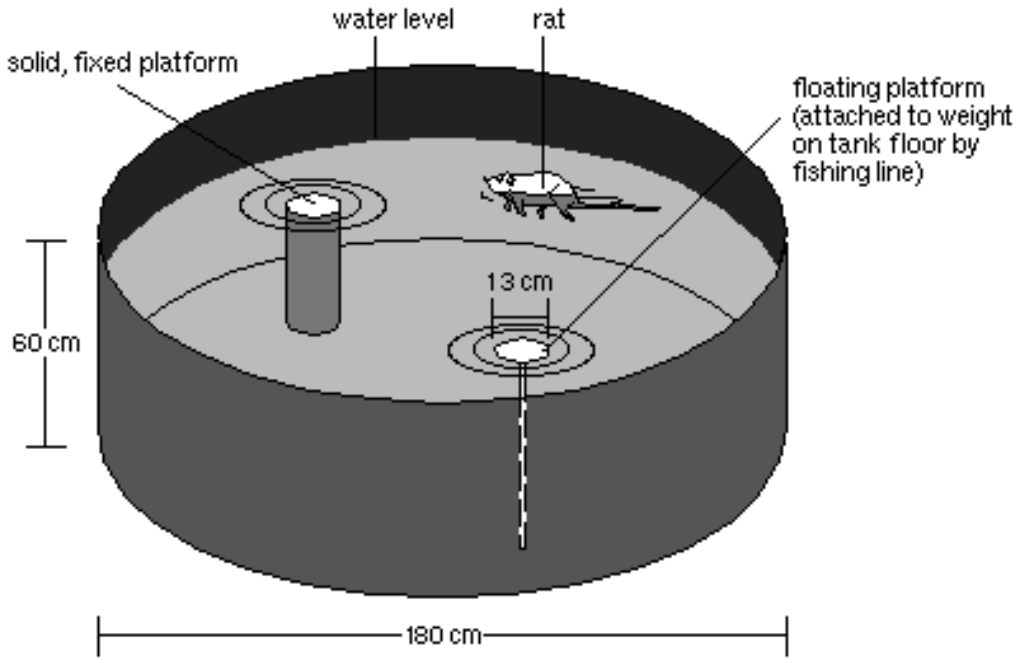

Figure 1. Illustrative diagram of Morris water maze (Decker, 2001).

Each group was composed of six mice, for a total of 30 mice. Each mouse was individually labeled and named in order to keep track of trends in the latencies recorded. Labeling was carried out via the use of picric acid, which was administered to the fur of one mouse of the pair living in a cage. Picric acid caused the portion the fur where it was applied to stain yellow, allowing the two mice in a cage to be distinguished from one another. In addition, the mice were arbitrarily named to permit specific identification when recording data. The names of the mice assigned to each group are listed as follows:

Group	Mice
Control	Mary, Kate, Stew, Drew, Tiger, Puma
Ammonium Hydroxide (1.12%)	Ren, Stimpy, Akmed, Muhammad, Pinky, The Brain
Methanol (2.5%)	Dexter, Dee-Dee, Usher, Ne-Yo, Omar, Khalif
Acrylamide (0.00000027%)	Donald, Jenna, Rocco, Erich, Tyga, Weezy
Cigarette Smoke	McDreamy, Meredith, House, Wilson, Walter, Jesse

The mice ran the maze a total of seven times over a period of 22 days (see Figures 2–6 in Results section). It was expected that exposure to the chemicals and smoke of cigarettes would induce a weakened ability to learn in mice, similar to the phenomenon observed in Alzheimer's patients. This reduction in recall ability should lead to greater latencies in the affected mice.

Animal Maintenance

30 female CF-1 strain mice were ordered from the Charles River Laboratory. The mice arrived at 12 weeks of age weighing approximately 32 to 37 g each. The experimental method required that the mice be kept in appropriate housing that is conducive to the application of the chemicals. The mice were housed in pairs in a standard-issue cage, with dimensions of 12 inches by 32 inches by 16 inches. The 30 mice were housed in pairs, thus 15 cages were in use. The mice had a supply of food and water from which they were able to eat at any time of day. The researchers and lab technicians checked on the mice periodically to ensure that ample food and water was available to the mice, and also to change the bedding in the cages.

Preparation of Chemicals

In order to decrease the chances of methodological error, all the chemicals were administered in the same manner. Each group of mice was exposed to its respective chemicals for a duration of three weeks, while intermittently being tasked with completing the Morris water escape task. The chemicals were mixed with the mouse's water in quantities proportionate to that which are found in Marlboro Red Full Flavor cigarettes. The values for the concentration of each chemical as contained in one cigarette were discovered to be: 11,160 parts-per-million of ammonium hydroxide, 3 mg of methanol, and 1.1 to 2.4 μg of acrylamide (Smith et. al, 2000). The ammonium hydroxide, methanol, and acrylamide were dissolved in water and placed in the drinking vessel. For ammonium hydroxide, the calculated concentration was 9.2 ml in 750 ml of water, yielding a 1.12% solution. For methanol, the concentration was 15.78 ml in 750 ml of water, yielding a 2.5% concentration. For acrylamide, the calculation was a lot more difficult to determine. This is because not only the amount of acrylamide found in a cigarette must be

considered, but also the extensive amount that is naturally excreted by the body due to its potency as a toxin. From Boettcher et. al (2005), it was determined that humans are exposed to approximately 3 μg per kilogram of body weight per day. This value was subsequently scaled down to the average weight of 12-week-old female mice. Based on the characteristics of the mice, it was estimated that each mouse would drink about 5.1 ml of water per day. Additional calculations revealed that each mouse should be exposed to 0.1378 μg acrylamide per day. This quota was attained by conducting a 2.7:1,000,000 serial dilution: 2700 μg of acrylamide was dissolved into 1 L water, then 1 ml of the resulting solution was diluted into another 1 L water. The resultant solution that was administered to the mice was a concentration of 0.00000027%. Although this number appears to be incredibly low, it is a feasible value when one takes into account the potent toxicity of concentrated acrylamide. The cigarette smoke was administered to the mice in the form of second-hand smoke from combusted cigarettes. First-hand smoke would have been ideal, but the resources in use did not allow for the mice to inhale the smoke directly from the cigarette. The setup consisted of a plastic chamber approximately 24 inches by 40 inches by 16 inches in which the 6 mice of the cigarette smoke group were placed. A cigarette was attached to a vacuum nozzle by vacuum tubing which served to create suction on the cigarette, resulting in the smoke being released into the chamber. The mice were exposed to smoke from 4 cigarettes for a period of 40 minutes twice per week.

Water Maze Specifications

The next step involved placing the mice in the Morris water maze that was constructed. The maze was constructed via the use of a 35-gallon steel tub 31.5 inches in diameter. The tub was filled with water to a level such that the walls of the tub rose approximately 18 inches above

the water line. The height of the walls and opacity of the tub were purposefully incorporated into the design to prevent the mouse from being distracted from external objects while navigating the maze. A height adjustable, circular platform was then placed in a consistent location of the tub, which was subsequently filled with water to a level 1 cm greater than the height of the platform. The mouse was then placed into the tub, back feet first followed by the front feet, to avoid stress. Also, the mouse was placed facing the wall of the maze to avoid bias to any one region of the maze (Barth, 1997). Milk was mixed into the water such that the water became cloudy and the mouse was unable to see the platform. The mouse was presented with the task of escaping to the platform by swimming. This water escape task is a good measure of a mouse's memory ability because quick, successful escape requires that the mouse be able to recall the areas it has already covered in reference to the starting location in order to locate the platform (Barron, 2010).

Microscopic Analysis of Amyloid Plaques

It was initially intended that upon conclusion of the experiment, the brains of the mice would be examined for the presence of amyloid plaques in the experimental mice. However, lack of sufficient resources and financial restraints prohibited this step from being carried out. The mice would have been euthanized via the use of carbon dioxide from a compressed gas cylinder to quickly and painlessly asphyxiate the mice. A dissection of the cranial region would then take place. The brain would be extracted and identical cross-sections of the cerebrum will be taken. The sections would be stained with congo red, according to the procedure carried out by Oakley et. al, 2006. Congo red reacts with beta-amyloid plaques to cause them to be visible. The sections would be analyzed under a light microscope and the number of plaques in each case would have been recorded.

CHAPTER SIX: RESULTS

The individual latencies were recorded in tabular form and then converted to graphical form. The graphs compare the individual latencies of the six mice in each group across the seven trials. In Figure 1, we can see that the latencies of the control mice were relatively high in the first week, especially when compared to the latencies of the other groups. This indicates that even prior to exposure to chemicals, the control group mice were slightly less adept at navigating the maze than mice of other groups. Efforts were made to ensure that there was an even distribution of mice according to skill. However, each group tended to have one mouse that was an outlier, which prevented complete equality of the groups from being achieved. For example, in the initial trial Puma was a lot slower than the rest of the control group, Muhammad was slower than the rest of the ammonium hydroxide group, Omar was slower than the rest of the methanol group, and Walter was slower than the rest of the cigarette smoke group. Only the acrylamide group did not have one mouse that was particularly slower than the rest. In theory, this near-equal spread of the outlier mice should have resulted in groupings that were approximately even.

In the control group, Puma served to an extreme outlier and was greatly responsible for the drastically high average latency of the control group. In the initial trial, Puma's latency was the highest by far of the group. Puma recorded an initial time of 100.8 seconds, whereas the next slowest time was 51 seconds. Therefore, Puma's time was almost twice as high as the slowest mouse of the other five in the group. This trend appeared again in later trials, most evident in the fourth trial: Puma recorded a time of 110.6 seconds, whereas the next slowest time was 11.7 seconds – almost ten times faster than Puma. Some of the reasons for Puma's data are mentioned in the Discussion section. However, Puma was not the only mouse who displayed abnormally

high latencies during the various trials. Almost all of the mice had one or two trials where they recorded an uncharacteristic time based on the progress they had been making in previous trials. Examples of this activity are Tiger, Drew, and Kate.

In the ammonium hydroxide group (Fig. 3), the overall trend is one of decreasing latencies. However, certain mice (Muhammad and Stimpy) were prone to sudden increases in latency. This trend is also seen in the methanol group (Fig. 4). In the case of the methanol group, both Omar and Khalif proved to be susceptible to the increases in latency. For the cigarette smoke group (Fig. 6), no one mouse in particular seemed to sporadically present an increase in latency. The data reveals that essentially all six of the mice had at least one trial where their latency increased significantly from the previous week. However, the latency would generally revert back to the expected latency for the next trial. Despite the trends observed in the previous four groups, the acrylamide group proved to display the most consistency in recorded latencies (Fig. 5). Excluding one abnormal latency from Rocco in Trial 3, the six acrylamide mice started off with low latencies and continually decreased these times throughout the seven trials.

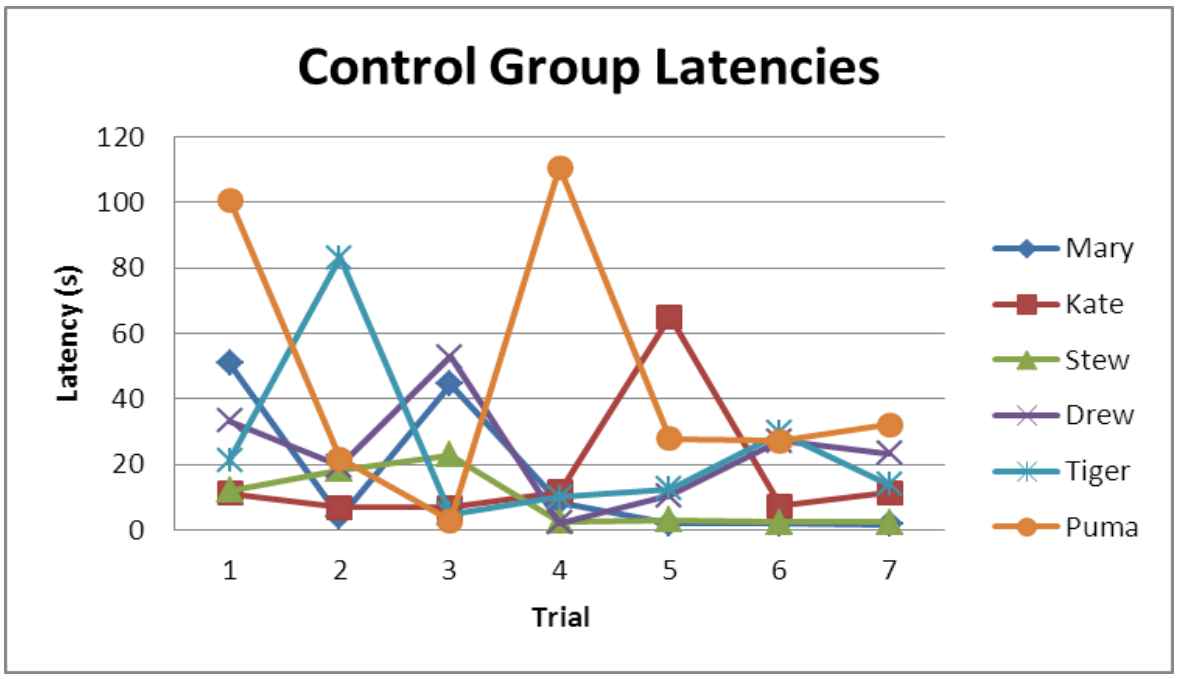

Figure 2. Latencies of the 6 control group mice as recorded in 7 trials over 3 weeks.

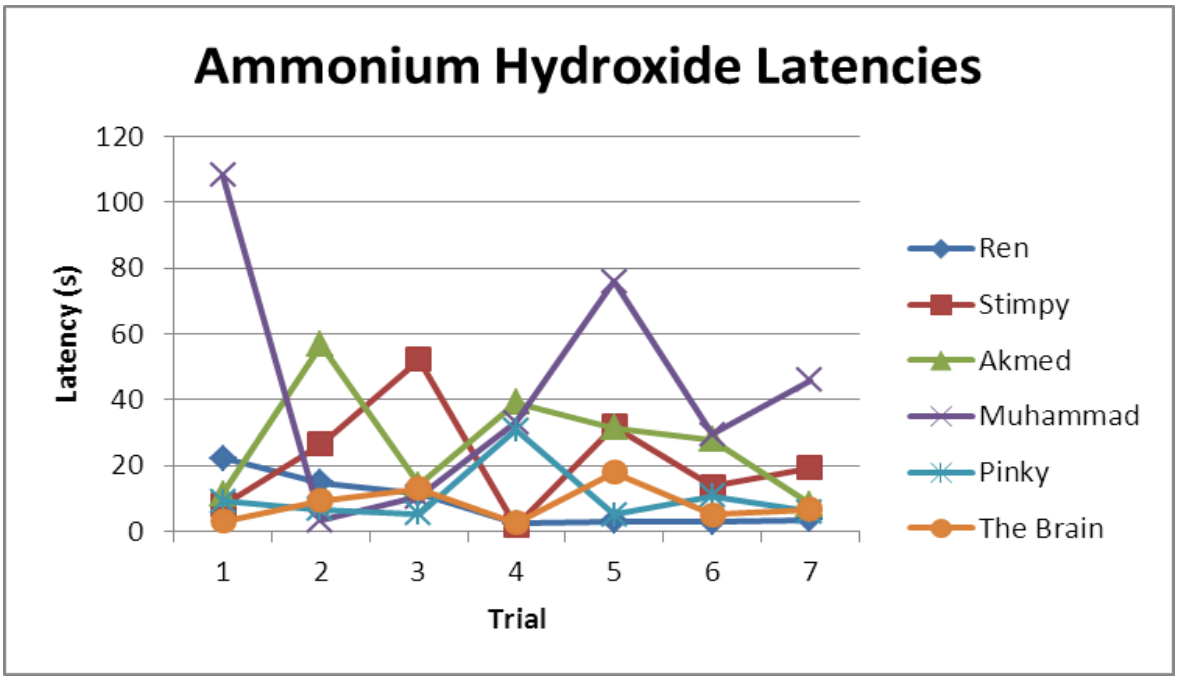

Figure 3. Latencies of the 6 ammonium hydroxide mice as recorded in 7 trials over 3 weeks

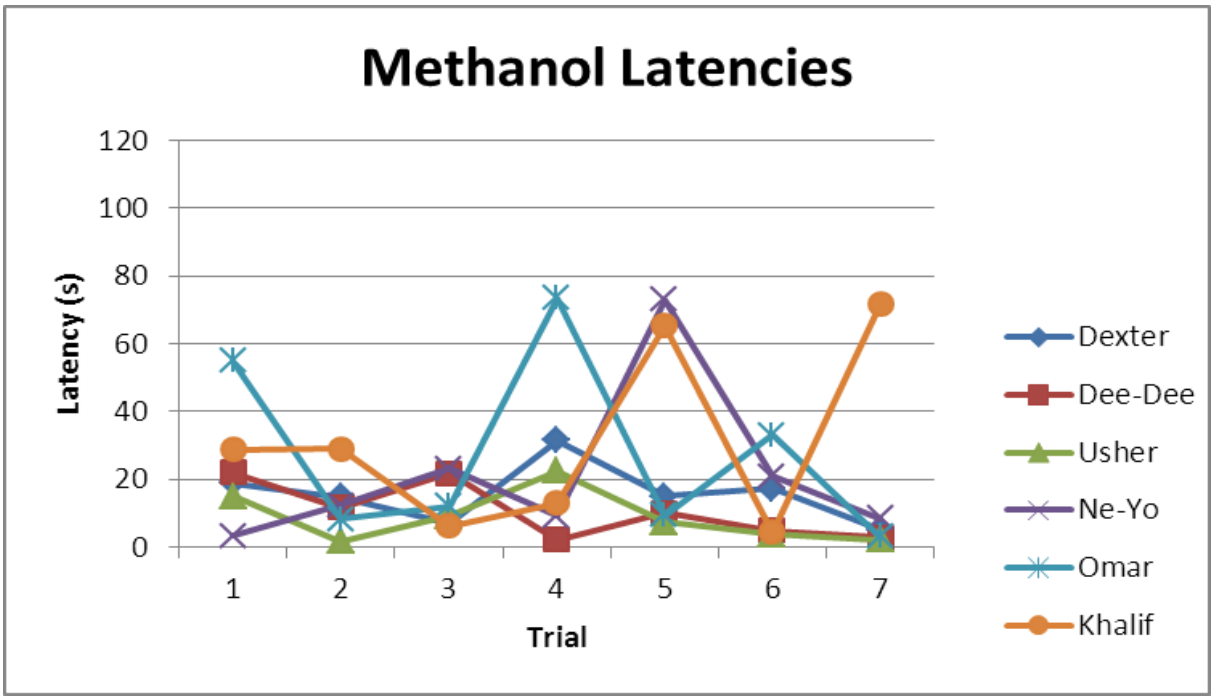

Figure 4. Latencies of the 6 methanol mice as recorded in 7 trials over 3 weeks.

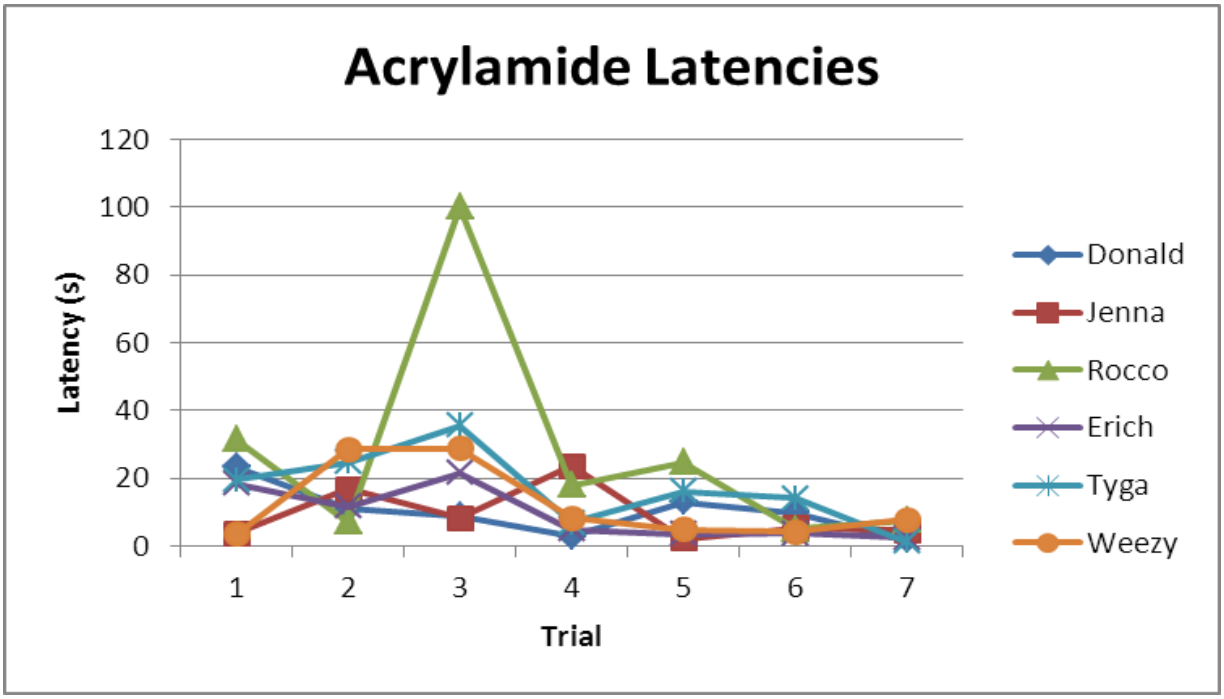

Figure 5. Latenices of the 6 acrylamide mice as recorded in 7 trials over 3 weeks.

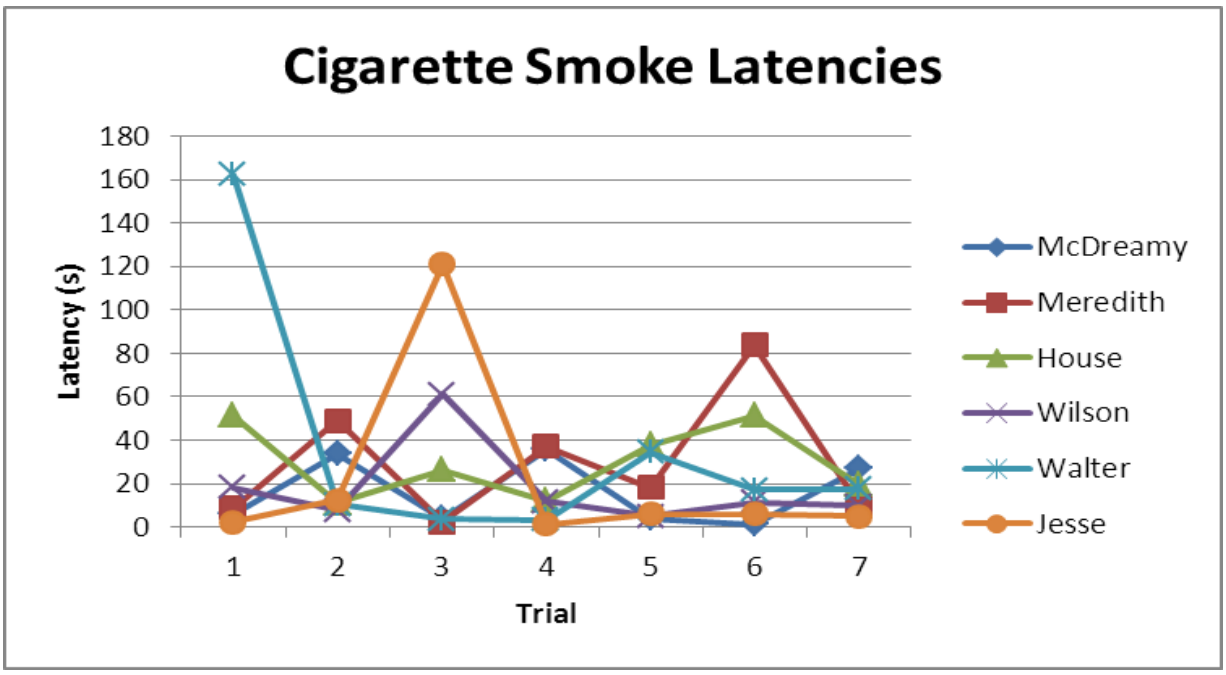

Figure 6. Latencies of the 6 cigarette smoke mice as recorded in 7 trials over 3 weeks.

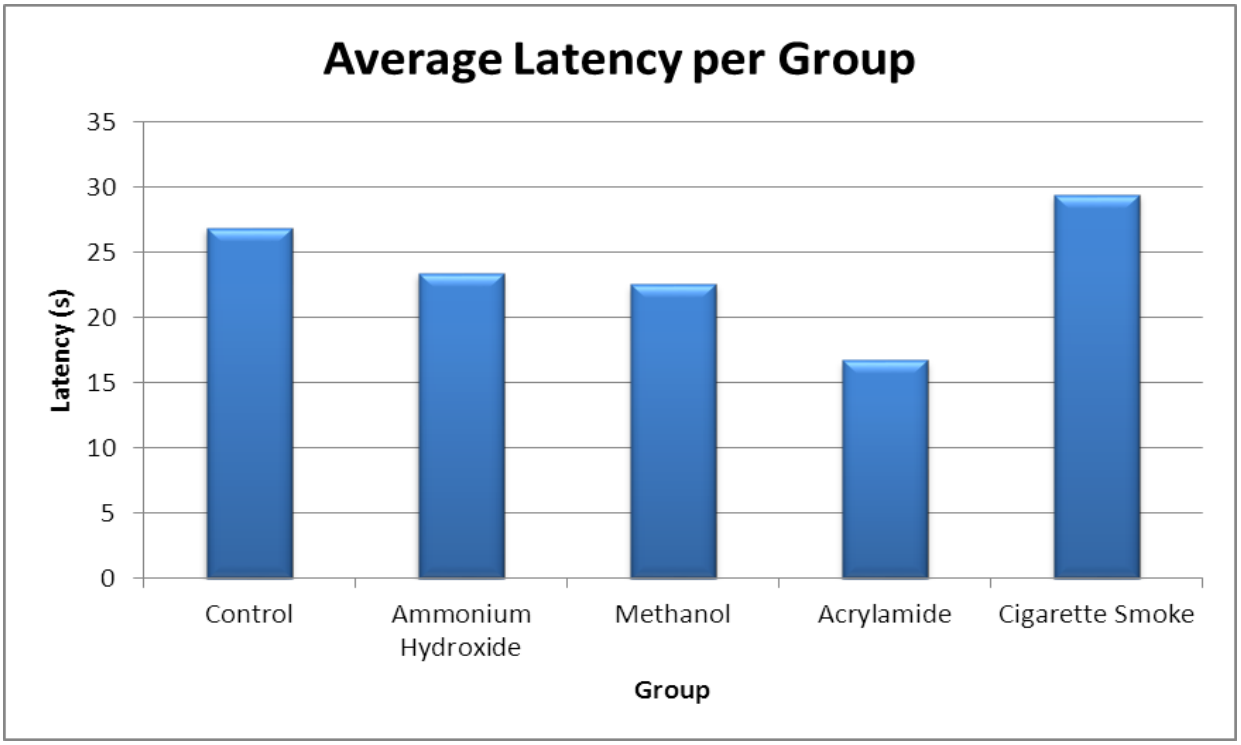

Figure 7. Average latency of each group of mice over the 7 trials.

A more detailed table was composed that displays the average latency of each mouse over the 7 trials, and then the average latency of the group as a whole (Table 1). From the table, it is clear that one mouse of the group generally had an overall average latency much higher than the rest of the mice in the group. This supports the earlier observation of the presence of an "outlier" mouse. The average latency of each group is listed in the table and also represented as a column chart in Figure 7. In graphical form, the relationship among the average latencies can easily be seen. Acrylamide was the fastest group overall, with an average latency of 16.7 seconds. Methanol was the second fastest, with ammonium hydroxide right behind at third. The control group was the fourth fastest, and the cigarette smoke group was the slowest overall.

Group	**Average latency of mouse over 7 trials (seconds)**						**Avg. latency of group (seconds)**
	Mouse 1	**Mouse 2**	**Mouse 3**	**Mouse 4**	**Mouse 5**	**Mouse 6**	
Control	19.0	20.1	10.7	28.3	29.3	53.9	26.9
Ammonium Hydroxide	3.5	19.4	8.3	46.0	6.1	6.7	23.4
Methanol	5.1	2.8	2.1	8.5	3.1	71.7	22.5
Acrylamide	1.8	4.7	7.6	2.5	1.4	7.8	16.7
Cigarette Smoke	27.0	9.8	19.4	9.7	17.6	4.9	29.4

Table 1. Average latencies of each mouse in each group, as compared to the average latency of the group as a whole.

From this data, it would by default appear that the inverse of our hypothesis was true: acrylamide performed the "best" and the control group was the second slowest. However, another method was devised to analyze each group's ability to learn. In Figures 8 to 12, the average latency of each of the five groups was recorded trial by trial. Then, a line of best fit was superimposed on the graph. The purpose of the line of best fit was to indicate the overall trend in the data across the seven trials. The equation of the line in slope-intercept form is $y = m\,x + b$, where m is the slope of the line. If the slope is negative, it indicates that the latencies generally decreased as time went on. The magnitude of the slope indicates the rate at which this decrease occurred. In addition, the coefficient of determination (R^2) is displayed on the graph. R^2 is a measure of how well the line of best fit actually matches the data of the graph. Comparison of Figures 8 to 12 reveal that although the control group posted the fourth best average latency overall, they displayed the greatest rate of learning as evidenced by a slope of $m = -3.36$. The cigarette smoke group had the next greatest slope with $m = -2.99$, and acrylamide third with $m = -2.84$.

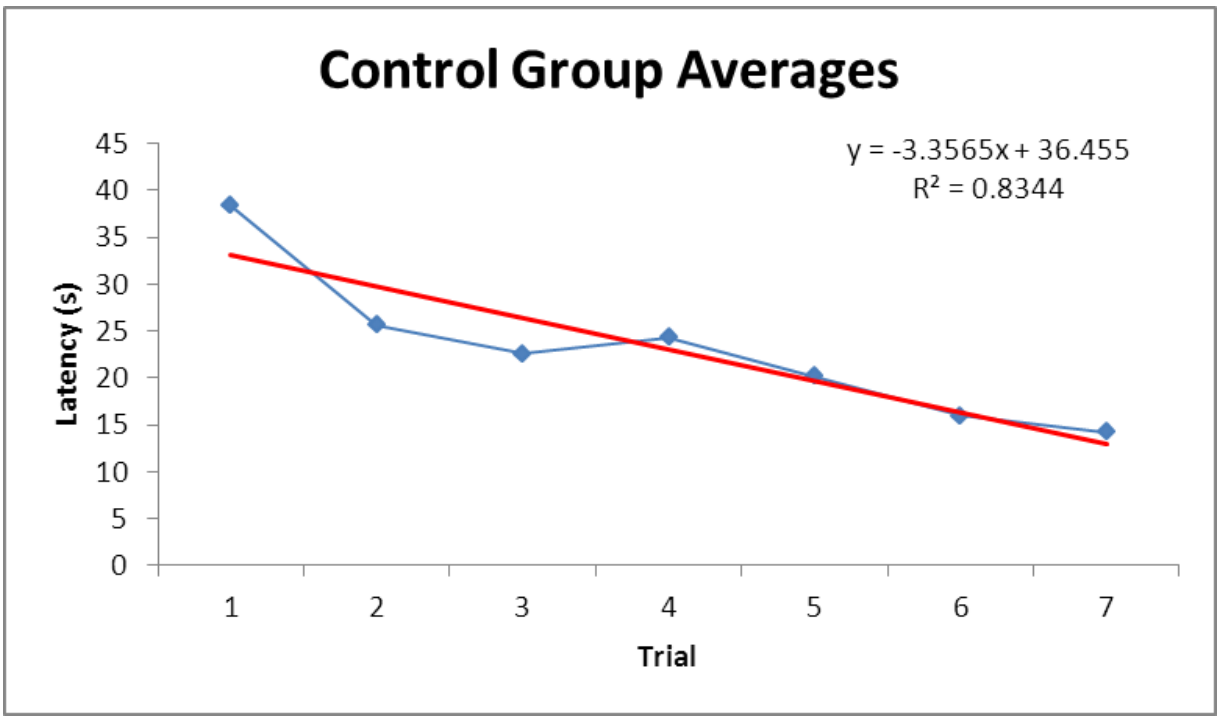

Figure 8. Average latency of control group by trial, with line of best fit added.

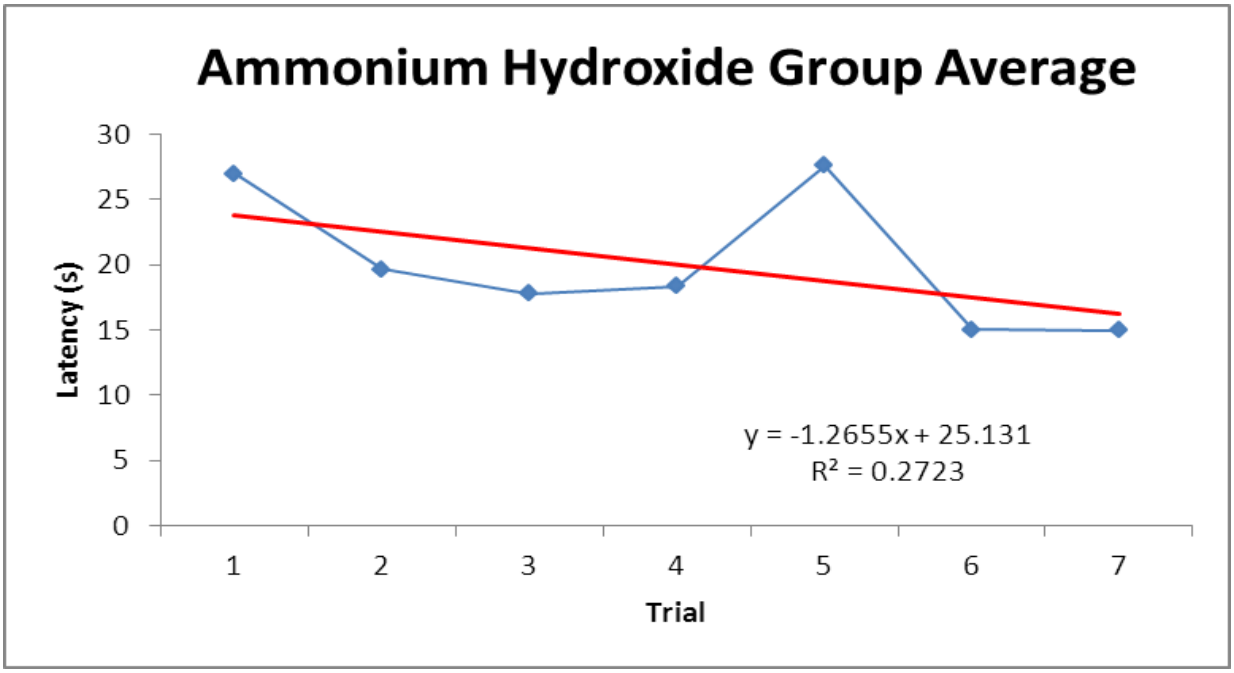

Figure 9. Average latency of ammonium hydroxide group by trial, with line of best fit added.

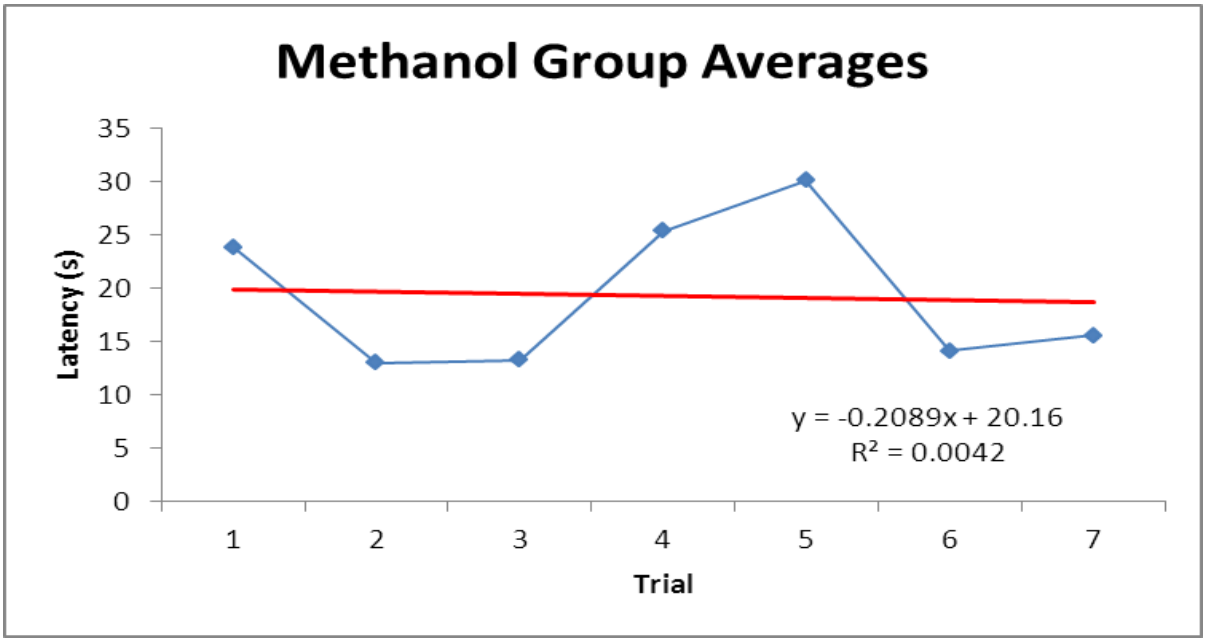

Figure 10. Average latency of methanol group by trial, with line of best fit added.

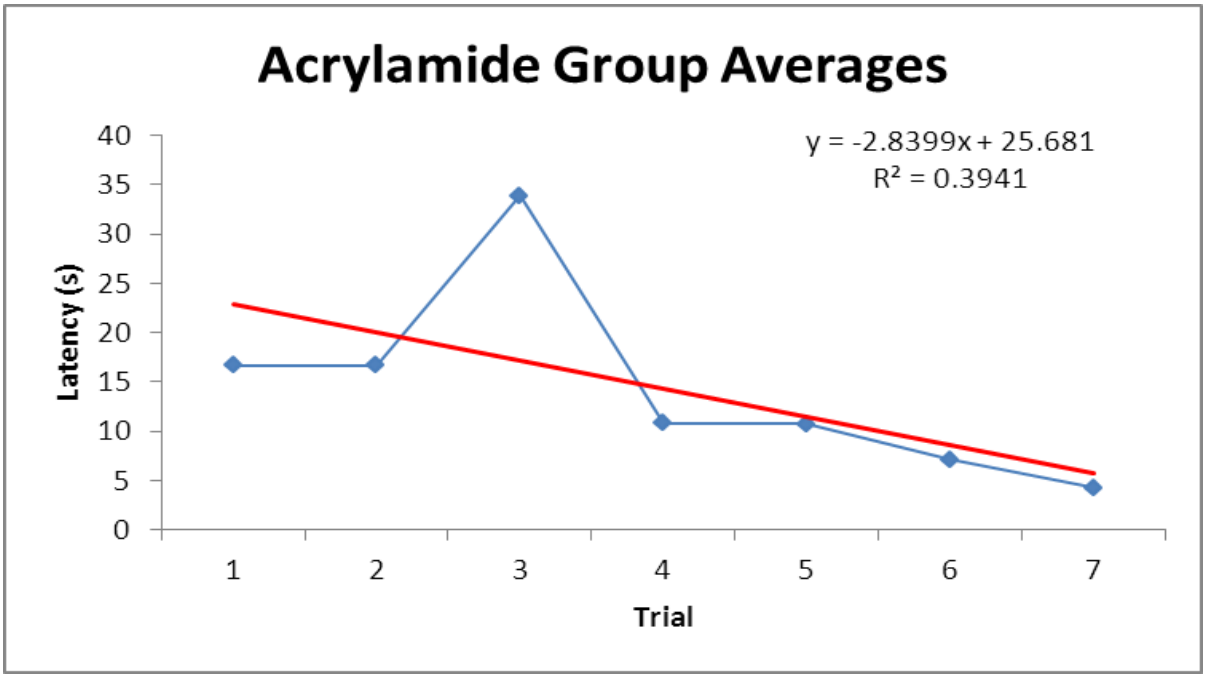

Figure 11. Average latency of acrylamide group by trial, with line of best fit added.

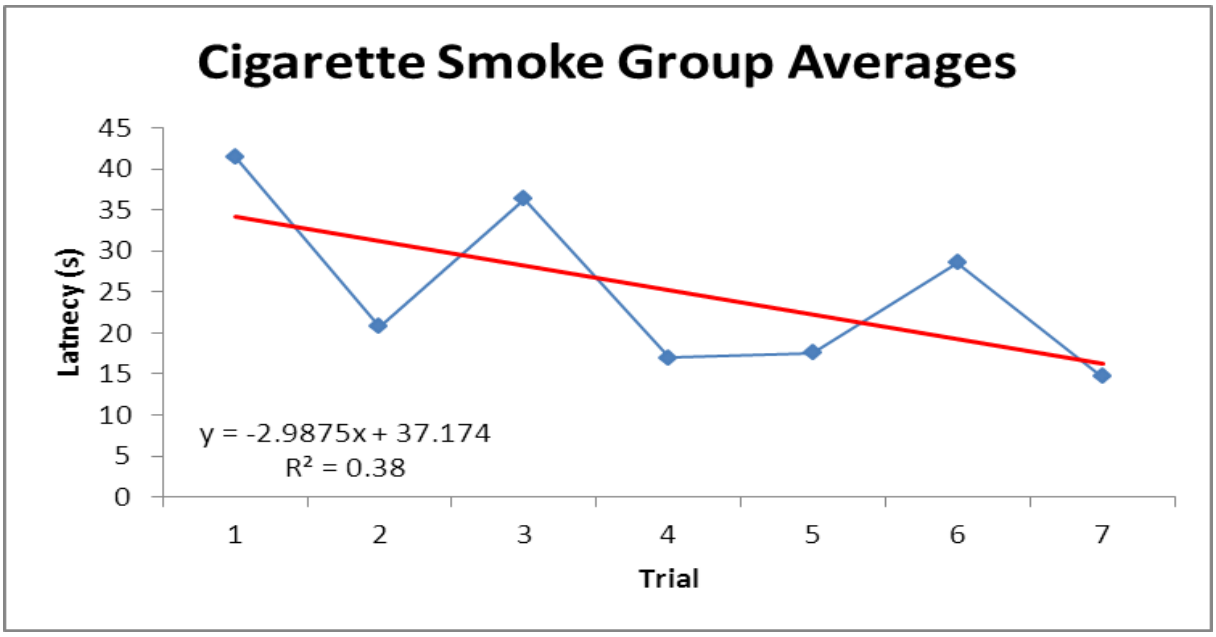

Figure 12. Average latency of cigarette smoke group by trial, with line of best fit added.

The average staying percentage of each mouse was also calculated (staying percentage is a representation of the mouse's tendency to remain on the platform or jump off once it comes into contact with it). These values are listed in Table 2.

Group	Average staying percentage over 7 trials (%)						Avg. staying percentage of group (%)
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	
Control	100	57.1	71.4	16.7	42.9	71.4	59.9
Ammonium Hydroxide	71.4	57.1	71.4	57.1	100	85.7	73.8
Methanol	57.1	28,6	85.7	100	85.7	85.7	73.8
Acrylamide	71.4	100	100	100	85.7	85.7	90.5
Cigarette Smoke	42.9	0	0	100	57.1	28.6	38.1

Table 2. Average staying percentage of each mouse, as compared to the group average.

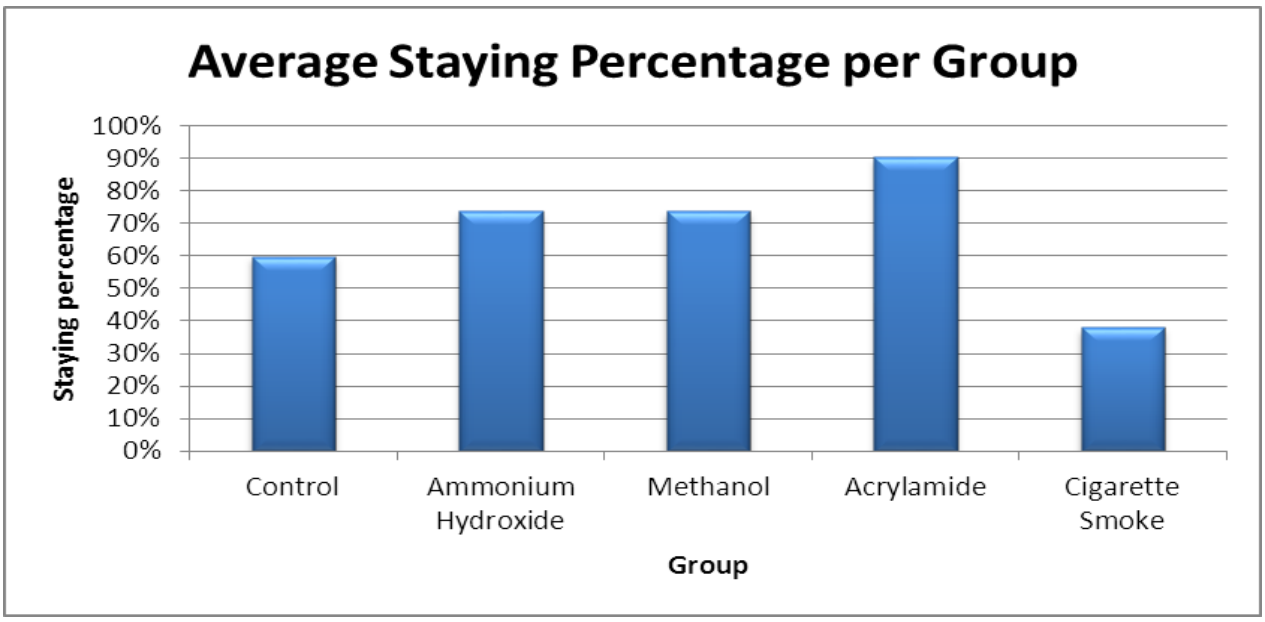

Figure 13. A graphical representation of the staying percentages of each group.

In addition to the experimentation with mice, we were able to distribute a survey to various members of the Saint Peter's University community (for a sample survey, see Appendix). After tallying the results, it was found that most people were not acquainted with any elderly people suspected to have dementia; therefore, their responses could not be considered and the results were inconclusive. Of the few people who knew of an elderly person with dementia, it was claimed that they did not smoke, or that the participant was unsure of their smoking habits. Therefore, we can conclude that this survey is an ineffective method to attain data on smoking histories of people who developed AD. However, surveys in general are an effective method to readily gather data from a wide range of participants, especially if the subject of the survey is more common. The fault in our survey lies in the fact that dementia is not a common pathology; therefore, we were unable to obtain a significant number of positive responses.

If we were to distribute another survey, we may change a few details in order to gather better results. First and foremost, it appears that our survey was too detailed: we were searching for specific information that was very unlikely to be found. Therefore, we might want to broaden the scope of the survey and the topics it inquires about. Many studies have pointed to the fact that it is the type-2 alkenes in cigarette smoke that cause the most damage to the nerve cells in the brain. Our analysis of studies has found that they are present in many other forms in the environment apart from cigarette smoke. Therefore, we can put questions on the survey that ask about the eating habits of elderly people that the participant is acquainted with (in order to examine the effects of French fries), and we can also ask for a description of the occupation the elderly person had for the majority of their life (to analyze exposure to chemicals in the workplace). Another way we can broaden the scope of the survey is to administer it to more people. Due to time restraints, we were only able to give the survey to selected students among the Saint Peter's community. This severely limits the range of people incorporated and prevents the survey from being distributed as a random sample. If possible, we would like to distribute the survey to people of other ages (both older and younger than the average college student), and to people residing in various locations in and surrounding Jersey City to ensure that as much diversity as possible is incorporated for the survey sample.

CHAPTER SEVEN: DISCUSSION

The results of our experiment have unexpected, though significant, implications. Firstly, the statistic that stands out the most is the average latency of 16.7 seconds for the acrylamide group of mice. Not only was this latency the lowest, it was lower than the rest by a substantial portion: the next closest group was almost four seconds slower. Thus, it would appear that this datum actually contradicts our initial hypothesis, that the presence of acrylamide in an organism would indirectly lead to a loss of memory. The faster time signifies that the memory of the acrylamide group was generally better than the rest of the mice. This is because for a mouse to have a low latency in completing the maze, it must learn over time where the platform is located relative to its starting position. Oftentimes, we found that the mice from the acrylamide group would head almost directly to the platform once placed in the water. The platform was located behind their starting position and they immediately knew to turn around and move in the general direction of the platform. Most times, the mouse's latency was less than 10 seconds. In addition, the mice would often swim in particular patterns that were good indicators that the platform was found via memory rather than by luck. Some examples of these are the circling swim patterns that some mice exhibited, the motion of swimming (reaching out with the extremities and tail to feel for the platform), and the greater amount of time spent in the quadrant containing the platform. However, before we simply assume that acrylamide is beneficial for the memory of mice, we must take other factors into account. An analysis of the first trial latencies reveals that the mice of the acrylamide group were inherently better at solving the maze than any other group. In this first trial, none of the mice had been exposed to chemicals, so they were all on a level playing field. Therefore, one could argue that it would be expected for these mice to do better because they were naturally more adept at solving the maze. Another concern is the

potentially low dosage of acrylamide we administered to the mice. The concentration, 0.00000027%, certainly seemed to be low, but due to acrylamide's high toxicity we decided to err on the side of caution rather than risk killing the mice. The result of this is that the prepared solution may have been too dilute to observe the effects on acrylamide on the mouse's ability to learn. In theory, the acrylamide mice may have been rendered another "control" group.

For the rest of the groups, other general trends were observed. For the most part, the mice in each group had slower times in the beginning and gradually brought down the total time it required to find the platform. However, there were usually a few mice that served as outliers and could not find the platform at the same rate as the others. One of these in particular was the mouse Puma from the control group. By the fourth trial, the rest of the control group were consistently recording low latencies. Puma, on the other hand, was posting abnormally high times because she would often "freeze up" once placed into the water. Specifically, she would not swim but merely hesitate in one region of the maze, not making any effort to find the platform. This behavior was noticed week after week and is simply attributed to a timid demeanor. Puma's consistent lack of vigor in the trials may have inaccurately increased the average latency for the control group.

After the acrylamide group, the next fastest group was the methanol group (approximately 22.5 seconds), the ammonium hydroxide group (23.4 seconds), the control group (26.9 seconds), and finally the cigarette smoke group (29.4 seconds). This order of latencies was again surprising because it displayed that the control group was only the fourth-fastest group. Both the methanol and ammonium hydroxide experimental groups managed to perform better than the control group. In addition, the swim patterns of the methanol group appeared to be very precise and deliberate. In contrast, some members of the control group appeared to be more

confused as they attempted to locate the platform. The one result that coincided with our expectations was the result of the cigarette smoke group. They had the slowest times but not by a great margin. However, it was noted that the mice from this group did not display any type of strategy when completing the maze. They appeared to simply swim around randomly until they came into contact with the platform.

In contrast with the conclusions obtained from the previous method of analysis, consideration of the line of best fit caused us to question whether average latency was a good indicator of mice's ability to learn. Average latency calculations suggested that acrylamide was the group that learned the best, while the control and cigarette smoke groups were fourth-best and last, respectively. However, according to the data from the line of best fit, the control group actually learned the best, followed by the cigarette smoke group, with the acrylamide group coming in third. These results somewhat coincide with our hypothesis, but certain aspects of the line of best fit's implementation prevent us from declaring the method to be a fully accurate indicator of learning. Firstly, the line is not always representative of the data in the graph. Only the control group had a line that correlated with the data over 50% of the time (83.4%). The ammonium hydroxide, acrylamide, and cigarette smoke group's lines ranged from 27.2% to 39.4% accurate. The line of best fit for methanol was extremely uncharacteristic of the data, with an correlation percentage of 0.004%. The other caveat with the line of best fit is the fact that it does not take into account the position from where the mice started when assessing learning rate. For example, mice that started off with high latencies will have a much easier time decreasing them as compared to mice that started off with low latencies. This is evident in the control and cigarette smoke groups, which had the greatest rate of learning but also started off with the

highest latencies. However, it is interesting to note that although the acrylamide group had the lowest average initial latency by far, it still managed to display the third fastest rate of learning.

Aside from latency, another statistic that was recorded was the behavior upon encountering the platform (see Table 2). The general expectation was that once a mouse located the platform, it would remain on it indefinitely due to the mouse's natural aversion to water. However, we noticed that although most mice acted in this manner, there was also a significant portion of mice that did not remain on the platform and instead opted to keep swimming. Therefore, the behavior of "stayed" versus "jumped" was listed alongside each mouse's latency to assess patterns in the platform behavior. Within this criterion, it was noted that different groups of mice were more likely than others to remain on the platform. The group with the greatest tendency to remain on the platform (i.e. "staying percentage") was the acrylamide group, which remained more than 90% of the time. It is unclear whether this tendency is related to their ability to complete the maze in exceptionally fast times or whether it is due to an extraneous variable. Two of the other experimental groups, ammonium hydroxide and methanol, had approximately the same staying percentages (73.8%). The control group had a lower staying percentage of approximately 60%. The group with the lowest staying percentage was the cigarette smoke group. This group's percentage was significantly lower than the rest of the groups at just slightly above 38%. Again, it is not apparent whether this significantly lower staying percentage can be correlated with the group's slower latencies, but the phenomenon is worthy of further study.

The results obtained from our study are not without potential limitations. The most prominent of these are the financial constraints and the duration of experimentation. After being denied a research grant, funding was a particular issue in regard to the materials we could obtain and the scope of our testing. Initially, we had intended to purchase more mice in order to be able

to examine the effects of greater numbers and concentrations of chemicals. The lack of funding severely restricted the testing we could do with the mice. In addition, greater financial resources would have permitted us to be able to purchase a professional Morris water maze. These mazes are built to more reliable specifications and also enable the researchers to more accurately track the mouse's swimming patterns. Although the maze that we constructed ourselves was sufficient to complete the task, it likely decreased the accuracy of our observations. The second major limitation in our research was the duration of the experiment. Due to a presentation deadline, we had specific time restraints and were unable to conduct experimentation for as long as we would have desired to. Three weeks is long enough to obtain preliminary data, but more concrete results would have been obtained if we were able to experiment for at least another 3 weeks. As a result of these conditions, we believe that if we had been enabled to conduct further testing we may have accrued more statistically significant data.

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APPENDIX

Table 3. Acrylamide levels in some processed foods.

Foods	Level of Acrylamide (μg/kg=ppb)
Roasted almond	260
Roasted asparagus	143
Bread, cake and cookies	70-140
Beer and malt	30-70
Biscuit and cracker	30-3200
Breakfast cereals	30-134 6
Chocolate powder	15-90
Coffee powder	170-351
Corn chips and cornflakes	34-416
Toast	800-1200
Fish products	30-64
Gingersnap	90-1660
Red meat and fowl products	30-64
Hazelnut and hazelnutoil	64-457
Husky peanut	140
Boiled potatoes	48
Potatoe chips and flakes	170-3700
French fryings	200-12000
Deep frying potatoes	1270
Other types of potatoe appetizer	30-1915
Roasted soybean	25
Roasted sunflower seeds	66

Figure 14. Percentage changes in selected causes of death (Alzheimer's Association 2012).

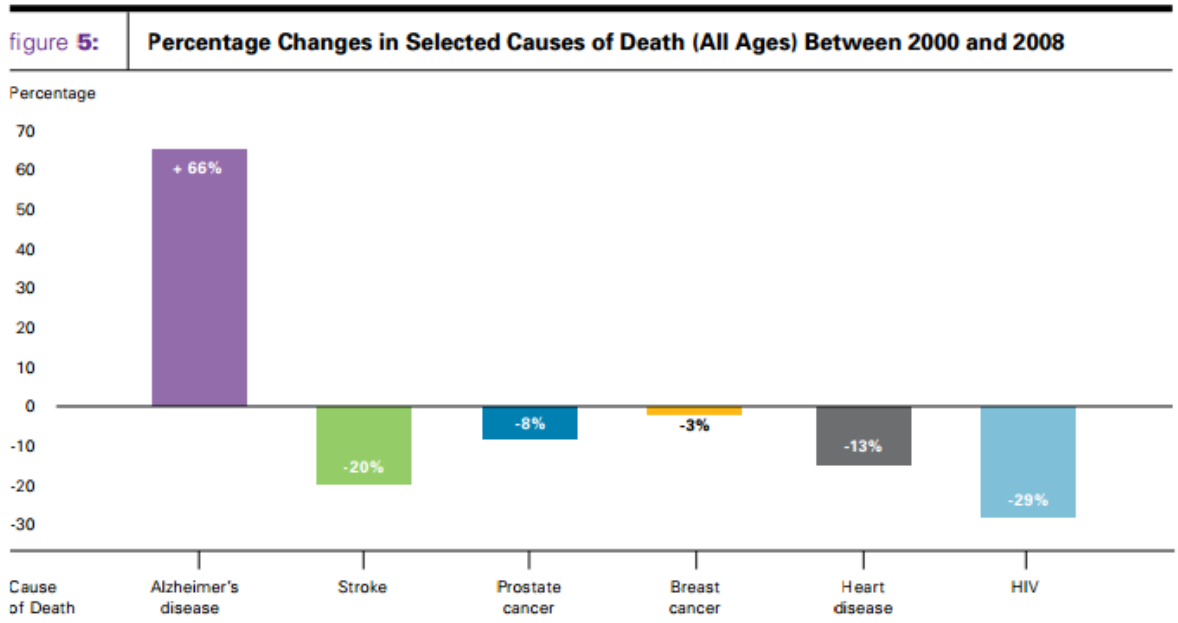
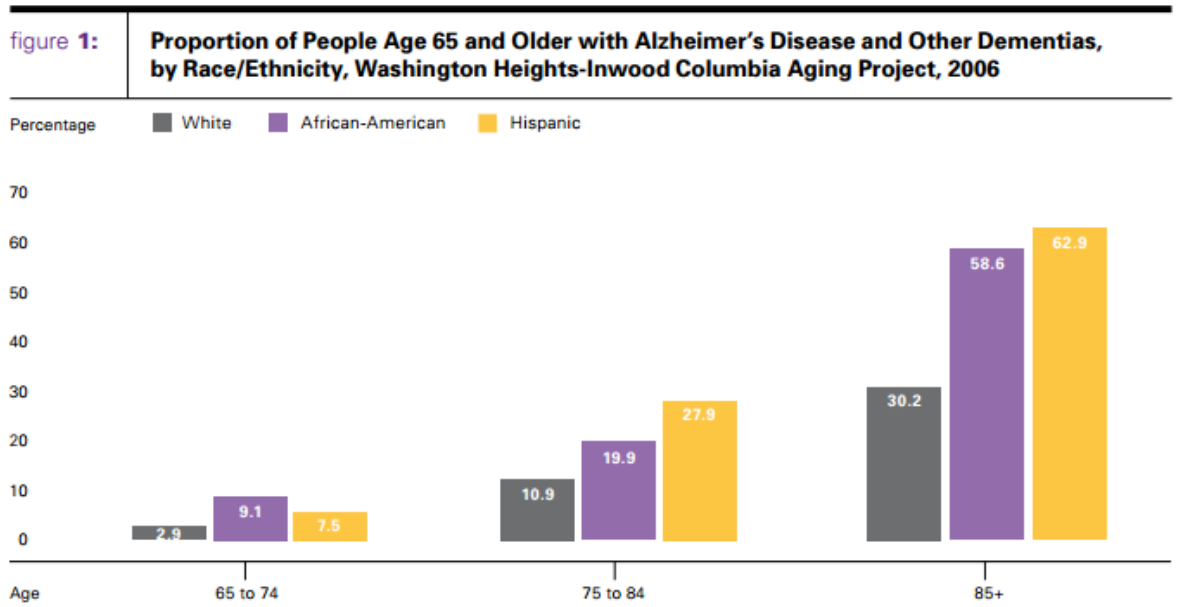


Figure 15. Proportion of people age 65 and older with Alzheimer's disease and other dementias, by race/ethnicity (Alzheimer's Association 2012).



An example of the survey distributed to members of the Saint Peter's University community.

1. Do you know of anybody aged 55 years and above who are current smokers, or have been smokers in the past?

   Yes No

2. If yes to Question 1, about how many cigarettes do they currently smoke (or previously smoked) per day? (one pack contains 20 cigarettes)
   A. Less than 1 per day
   B. 1 to 5 per day
   C. 6 to 10 per day
   D. 11 to 20 per day
   E. More than 20 per day
   F. Not sure

3. If yes to Question 1, around how long have they smoked (or previously smoked)?
   A. Less than 5 years
   B. 5 to 10 years
   C. 11 to 20 years
   D. 21 to 30 years
   E. More than 30 years
   F. Not sure

4. Of these smokers referred to above, do you currently know of (or have known of) any who have been diagnosed with dementia? (Dementia is a loss of brain function that affects memory, thinking, and behavior)

   Yes No

5. If so, what type of dementia were they diagnosed with?
   A. Alzheimer's disease
   B. Vascular dementia
   C. Creutzfeldt-Jakob disease
   D. Other: _____
   E. Not sure

6. To your knowledge, how many members of your family (immediate or extended) have been diagnosed with dementia? If yes, list the type(s) in the space provided
   A. None
   B. 1 family member
   C. 2 family members
   D. 3 or more family members

   Type(s): _____