THE EFFECTS OF ASPARTAME ON MEMORY IN RATS

By

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Introduction

Aspartame (1-methyl N-L-a-aspartyl-L-phenylalanine) is a low calorie sweetener that has been trademarked by multiple companies including NutraSweet, NutraTaste and Equal (Shapiro, 1988). Aspartame is about 180 times sweeter than normal sugar and is used in over 5000 foods and beverages in today's market. Consumer reports have shown that over 200 million people have consumed Aspartame world wide (Shapiro, 1988).

In 1965 Aspartame was discovered by accident by James Schlatter of the G.D. Searle Company. G.D. Searle noticed aspartame for its sweetness and began to run tests on this substance to check for safety and explore possible uses (Garriga & Metcalf, 1988). In July of 1974, Searle first petitioned the FDA (Food and Drug Administration) for approval of the use of aspartame in dry food products and beverages. At this time aspartame was approved for products including pudding, gum, breakfast cereal and use as a table top sweetener.

Multiple issues about the safety of aspartame arose prior to the FDA approval in 1974. During this time the FDA agreed that further evaluation of aspartame was needed before the product could be approved (Council on Scientific Issues, 1985). Most of the safety concerns arose due to the questionable practices by Searle while they were testing aspartame. In 1974, Searle submitted 15 studies to the FDA, out of which three were questioned due to possible unethical practices. Due to these three studies the FDA expressed concern that aspartame was more toxic than Searle reported in their findings. The three questionable studies were questioned on the basis of reported laboratory practices and potentially invalid

results. According to Searle, all three studies reported that aspartame was safe and did not cause brain tumors or other adverse health problems in rodents (Council on Scientific Issues, 1985). When the FDA requested clarification of these three studies, Searle sent a different set of data to the FDA that erased data points that were potentially negative (Millstone, 1994). Upon closer investigation of the G.D. Searle Company by the FDA, it was discovered that they had concealed multiple cases of brain tumors in the aspartame fed animals, data points were altered, and the testing process and diet had varied within each group (Millstone 1994).

Independent researchers began to investigate the safety of aspartame as a food additive. Research conducted by Olney and Ho (1970), showed that when an oral dose of aspartate was given to young mice it has a neurotoxic effect. The FDA launched an investigation of aspartame with two major concerns (Council on Scientific Issues, 1985). The first concern was that the consumption of aspartame led to mental defects and/or neuro-endocrine disturbances by causing brain damage. The second major concern was that aspartame was a cause of some brain tumors (Council on Scientific Issues, 1985). An FDA appointed task force and a Public Board of Inquiry (PBOI) evaluated the studies Searle had submitted to the FDA and led an investigation into potential health risks of aspartame. The task force reached the conclusion that aspartame was not likely a significant health risk but more testing was necessary to substantiate its safety. The PBOI also found that there was no significant risk for brain damage or mental defects from aspartame consumption; however, it was not able to reach a conclusion about the risk of brain tumors. They

also recommended further studies before final approval (Millstone, 1994; Olney 1988).

In spite of the recommendations by the FDA's task force and the PBOI, aspartame was approved by the FDA commissioner for use in dry foods and beverages in July of 1981 and in carbonated beverages in 1983. Aspartame was approved for use as a general purpose sweetener that could be used in any beverage or food in 1996. Today it is used in over 5000 products worldwide which include baked good, cereal, fruit-based spreads, gelatin, cereal, snack food, and beverage products (NutraSweet®, 2008).

Consumption of Aspartame

A Post-Marketing Survey (PMS) was used to monitor the average amount of aspartame consumed by the public from the time of its approval until 1987. The PMS was used to compare actual consumption of aspartame to the acceptable daily intake value (ADI). The ADI that is currently set in the United States is 50 mg/kg of body weight. A dietary survey of the PMS found that about 35% of the 2000 households examined regularly consumed aspartame (FDA, 1988). This survey also found that the average amount of aspartame consumed per day per individual was about 50 mg, corresponding to less than 1 mg/kg of body weight and less than 2% of the ADI for the individual. The survey also found that the high-level consumed was about 4% ADI based on body weight. Using these figures the FDA has said that aspartame is being consumed in levels well below what would be considered toxic (FDA, 1988). Consumption of aspartame in the United States has risen in recent years. The sales of aspartame in the United States have risen from

3,300 metric tons in 1985 to 10,100 metric tons in 2002 as reported by the CEH Marketing Report. Other reports show global production of aspartame at 18,000 metric tons with about half of that consumed in the U.S. in 2006 (Magnuson, Burdock, Doull, Kroes, Marsh, Pariza, et al., 2007). Diet soda is the most common form of consumption of aspartame. The consumption of aspartame, in diet soda, has risen from about 4.8 ounces per day in 1984 to 5.5 ounces per day in 2004, with most of the increase coming from 2002-2004 (Magnuson, et al., 2007). The average consumption of aspartame in the general population was calculated to be 3 mg/kg per person per day when various studies of general global populations and special subgroups were considered (Magnuson et al., 2007).

Many researchers believe that these numbers are too conservative. For example, Pardridge (1988) found that children consumed 50-77 mg/kg of aspartame a day when given free access to aspartame containing food and beverages. Children who consume only five servings of products containing aspartame consume about 34 kg/mg daily, placing them in the 99th percentile as established by the Council on Scientific Affairs. As hundreds more aspartame containing products have become available, the consumption of aspartame in the average household may have increased to a point substantially above the FDA's estimate (Pardridge, 1986).

Adverse Reactions to Aspartame

Since the approval of aspartame, 40% of all complaints issued to the FDA have been concerning adverse reactions after consumption of aspartame (Young, 1988). A variety of results have been found when aspartame was tested in both human and animal models. Some of these studies, along with anecdotal evidence

and case studies, have reported adverse reactions that could be attributed to the additive aspartame (Walton, 1986; Orange, 1998; Watts, 1991; Koehler & Glaros, 1988). A recent study found that female rats fed aspartame developed more lymphomas and leukemias than controls, in a dose-dependent manner, starting from a dose that may be relevant to human intake (20 mg per kg body weight), which is lower than the acceptable daily intake established by the Food and Drug Administration at 50 mg per kg body weight (Soffritti, Belpoggi, Esposti, & Lambertini, 2005) Another study found that when aspartame was administered to male and female Sprague-Dawley rats from the 12th day of fetal life until natural death there was a significant dose-related increase of malignant tumors in the male animals. Researchers also found a significant increase in incidence of lymphomas/leukemias in males and females, and significant dose-related increase in incidence of mammary cancer in females (Soffritti, Belpoggi, Tibaldi, Esposti, & Lauriola, 2007). In males, the most frequent histotypes observed were lymphoimmunoblastic lymphomas that mainly involved lung and mediastinal/ peripheral nodes. In females, the most frequent histotypes were lymphocitic lymphomas and lymphoimmunoblastic lymphomas that mainly involved the thymus, lung, spleen, and peripheral nodes (Soffritti et al., 2007). Conversely, some studies have shown no adverse consequences of aspartame consumption (Tilson, Hong & Sobotka, 1991; Spiers *et al*., 1998). The differences between these two groups may be due to multiple factors including subject expectations, influence of industry funding, and brief studies that fail to look at long term results (Olney, 1988; Leon *et al*., 1989; Kulczycki, 1995).

Also differences in the specific type of aspartame used in a study may cause a disparity in results of the research. Multiple studies have used 98% pure aspartame that has been provided by the NutraSweet Company; however, the aspartame consumed by the public is not as pure due to changes in temperature and aging of the sweetener (Kulczycki, 1986). The reports that cite aspartame as the cause of adverse health reactions maybe due to the degraded aspartame purchased by consumers.

Even with no common conclusion among researchers, aspartame has been listed as the cause of over 90 different adverse health reactions. Some of these reactions include cognitive and memory impairments (Orange, 1998), urticaria (Kulczycki, 1986), mood disturbances (Walton, 1986), and other reactions. It has been found that two-thirds of such reactions involve neurologic or behavioral symptoms (Morbidity and Mortality Weekly Report, 1984). The majority of these symptoms and complaints have been reported to the FDA or the NutraSweet Company. The FDA uses a passive surveillance system to record complaints and has a division called the Adverse Reaction Monitoring System (ARMS) that will investigate serious concerns that have been reported (Tollefson & Bernard, 1992).

Metabolites of Aspartame

Approximately 50% of the aspartame molecule is phenylalanine, 40% is aspartic acid, and 10% is methanol (Newsome, 1988). Large doses of both aspartame as well as these individual metabolites have been tested in humans and other animals. However because the rate of metabolism of these substances varies from species to species, the results are not always comparable. For example, rats

have a much higher metabolism for both phenylalanine and methanol than humans, and it is difficult to produce toxicity from either of these two components in rats (Fernstrom, Fernstrom, & Gillis, 1983; Stegnik, 1987).

The research on possible mechanisms of adverse neurological reactions to aspartame has focused on three major areas (Fernstrom, Fernstrom, & Gillis, 1983). The first concern is whether aspartame increases blood and plasma levels of aspartate, phenylalanine, and methanol, all of which are known to be neurotoxic in certain conditions. The second concern deals with the byproducts created by degraded commercial aspartame that are the result of temperature changes and time prior to usage. The byproducts have also been examined to study their possible negative effects. The final concern is whether levels of other large neutral amino acids, tyrosine and tryptophan, change after consumption of aspartame (Fernstrom, Fernstrom, & Gillis, 1983)

Aspartic Acid

Aspartic acid is a metabolite of aspartame that is an excitatory amino acid and is normally found in high levels in the brain (Maher & Wurtman, 1987). These levels are controlled by the blood-brain barrier which protects the brain from large fluctuations in plasma aspartate (Maher & Wurtman, 1987). Previous finding have shown that aspartate may lead to neurotoxicity through sustained contact with the receptors, such as glutamate producing an excitotoxic effect (Olney, 1990). Research has also shown that a dose of aspartic acid may exacerbate certain neurological conditions such as neurodegenerative disorders (Alzheimer's disease) and epilepsy (Dawson, 1988). It is still not determined whether aspartame

consumed in both normal and high levels is enough to cause a substantial increase in aspartate in the brain to produce neurotoxic effects (Stegnik, 1987).

Phenylalanine

The metabolite phenylalanine that is present in aspartame is considered to be an unresolved safety issue. Phenylalanine is an amino acid essential to the production of monoamines in the brain and is found in nearly all protein foods (Maher & Wurtman, 1987). Phenylalanine is quite commonly ingested but the body contains no mechanism for regulating the levels of phenylalanine in the body (Maher & Wurtman, 1987). According to the Council on Scientific Affairs (1985) when the levels of phenylalanine reach 100 mmol/dL or higher in an adult brain damage will occur; however, an individual's threshold may depend on other conditions, for example pregnant women have a toxic threshold of 50mmol/dL.

Phenylalanine consumption has also been seen to be hazardous to individuals who suffer from phenylketonuria (PKU). PKU results from a recessive genetic disorder where the individual does not have phenylalaylase and cannot convert phenylalanine into tyrosine (Garriga & Metcalfe, 1988). Due to this lack of an enzyme, these individuals can have extremely high levels (120-200 mmol/dL) of phenylalanine in their blood and without preventative treatment from a young age, the high levels can produce mental retardation (Stegink, 1987). Due to the high levels of phenylalanine in their blood the consumption of aspartame may cause brain damage (Stegink, 1987).

Methanol

The final major component of aspartame is methanol and only accounts for 10 percent of the aspartame molecule (Newsome, 1988). Methanol is quickly absorbed into the gastrointestinal tract upon consumption (Garriga & Metcalf, 1988). The methanol is then converted into formaldehyde and then into formic acid and excreted from the body (Garriga & Metcalf, 1988). The body also receives methanol from sources such as fruit and vegetables (Stegink, 1987). Previous research has shown that high levels of methanol can produce toxicity and cause seizures and blindness (Stegink, 1987).

Byproducts of Aspartame

Another concern in aspartame research is the consumption of the byproducts of aspartame. The degradation of aspartame is due to both temperature changes and the spent time spent before usage. Diketopiperazine (DKP), aspartyl-L-phenylalanine, a further increase in methanol (Lipton, Li, Younoszai, & Stegink, 1991), D-aspartic acid, and D-phenylalanine (Bohem & Bada, 1984) can be formed after exposure to heat, whether during shipping, baking, or in heated foods or beverages (Council on Scientific Affairs, 1985). In the testing of aspartame by G.D. Searle, DKP was looked upon as a possible tumorigenic agent (Millstone 1994). Researchers found that 6 months after aspartame was put into carbonated beverages, 25% of the aspartame had been converted to DKP (Tsang, Clarke, & Parrish, 1985). Concern among some scientists has been expressed that this form of DKP would undergo a nitrosation process in the stomach producing a type of chemical that could cause brain tumors (Tsang, Clarke, & Parrish, 1985; Shephard,

Wakabayashi, & Nagao, 1993). A one day exposure study showed that the DKP was tolerated without adverse effects (Geha, Buckley, Greenberger, Patterson, Polmar, Saxon, Rohr, Yang, & Drouin, 1993). Many researchers believe that DKP needs to be tested further to see the possible negative effects of aspartame consumption.

Methanol is primarily oxidized in several tissues to formaldehyde and formic acid (Murray, Burton, Rajani, Lewandowski, Burke, & Eells, 1991). Formaldehyde is a highly reactive small molecule which strongly binds to proteins (Haschemeyer & Haschemeyer, 1973) and nucleic acids (Metzler, 1977) forming adducts which are difficult to eliminate through the normal metabolism pathways. As a result, formaldehyde induces severe functional alterations (Heck, Casanovam & Starr, 1990), including the development of cancer (Blair, Saracci, Stewart, Hayes, & Shy, 1990). The small amounts of formaldehyde which can be potentially produced from dietary use of aspartame has been often overlooked in its potential toxicity precisely because of the limited amount produced (Troche, Pardo, Rafecas, Virgili, Remesar, Fernandez-Lopez & Alemany, 1998).

Neurotransmitter Changes

Another proposed mechanism for adverse neurological and behavioral reactions is an alteration in neurotransmitters resulting from the phenylalanine portion of the aspartame molecule. Phenylalanine is converted by phenylalanine hydroxylase in the liver to tyrosine. Tyrosine is a precursor to the formation of dopamine, norepinephrine, and epinephrine. When there is an increase in plasma tyrosine, there are corresponding increases in brain tyrosine levels (Torii, Mimura,

Takasaki, & Ichimura, 1986). Tryptophan, a precursor to serotonin (5-HT), is determined by the levels of large neutral amino acids (LNAA) such as leucine, isoleucine, valine, tyrosine, and phenylalanine (torii et al., 1986). LNAA's share the same transport mechanism into the brain and compete for uptake. According to Fernstrom and colleagues (1983), large levels of phenylalanine may reduce the transport of other LNAA's at the same transport site into the brain and thus affect the formation of several neurotransmitters including dopamine, norepinephrine, and 5-HT.

Aspartame Consumption and Memory

Previous research concerning aspartame consumption and memory have conflicting results. Some research asserts that aspartame has no effect on memory or learning (Tilson, Hong & Sobotka, 1991; Lappierre et al., 1990; Spiers et al., 1998). According to Tilson and colleagues (1991), rats were given acute intragastric administration of aspartame for 14 days. Researchers found no effect on spatial and reference memory in the Morris Water Maze (MWM) (Tilson et al., 1991). Conversely, several studies contend that aspartame does have an effect on learning and spatial memory (Potts, Bloss & Nutting, 1980; Dow-Edwards, Scribiani & Riley 1989, Christian et al., 2004). According to Christian and colleagues (2004), when rats received aspartame for 3-4 months they took significantly longer to find the reward in the t-maze. Another study found that when aspartame was administered to weanling rats as 9% of the diet (about 11 g/kg/day) for thirteen days it altered the learning behavior of male rats (Potts, Bloss & Nutting, 1980). Previous research has also found that aspartame exposure at 500 mg/kg throughout gestation

disrupts odor-associative learning in 15-day-old guinea pigs (Dow-Edwards, Scribiani & Riley 1989). Human literature also supports the hypothesis that aspartame may contribute to memory impairments. According to Orange (1998), students voluntarily participated in a preliminary study that involved drinking soda and performing memory tasks. The participants were randomly assigned to five groups of twelve students each. The comparison group did not drink any soda and each of the four experimental groups who drank one of the following types of soda: regular cola, regular caffeine-free cola, diet cola, and diet caffeine-free cola. Participants, who did not drink any soda, performed significantly better on the memory tasks than the soda drinkers and the diet-caffeine-free cola group had the poorest performance on the memory tasks (Orange, 1998).

Studies of aspartame in the peer reviewed medical literature were surveyed for funding source and study outcome. Of the 166 studies felt to have relevance for questions of human safety, 74 had Nutrasweet industry related funding and 92 were independently funded. One hundred percent of the industry funded research attested to aspartame's safety, whereas 92% of the independently funded research identified a problem (Walton, Hudak, Green-Waite, 1993).

The Present Study

In the present study, rats were fed aspartame for a 3 month period and their spatial memory was tested in the radial arm maze. Researchers hypothesized that rats who received aspartame would commit more memory errors than rats that were not fed aspartame. It is also hypothesized that animals who receive aspartame will also commit more long-term memory errors than the control animals. Our final

hypothesis was that the aspartame animals will have fewer neurons in the medial arcuate nucleus than control animals. The arcuate nucleus is an area particularly vulnerable to glutamate induced damage due to its proximity to a cumventricular organ (CVO) (Olney & Ho, 1970).

Methods

Subjects

Twenty-eight male Long-Evans hooded rats (Fourteen from the Texas Christian University breeder; fourteen from Harlan Sprague Dawley, Inc) were used in this study. Rats were gently handled prior to testing. Housing standards were maintained according to USDA protocol. All animals were individually housed in hanging plastic cages and were maintained in standard 12/12 hr light/dark cycle. At 90 days of age animals were randomly divided into 2 groups: Control (n =14) and experimental (n=14). Animals were then placed on a food restricted diet with free access to water.

Food Regimen:

At 90 days of age animals were randomly assigned into two groups, the experimental group received 6.5 grams of Equal (aspartame plus maltodextrin) daily and the control group which received 6.5 grams of maltodextrin. Both groups received this dose in 2.5 grams of cookie dough. The cookie dough and the Equal or maltodextrin was combined using 2 ml of water making a slurry. This was done to ensure that each subject received the same dose of aspartame or control substance. Aspartame or maltodextrin administration began on the first day of

behavioral testing. All animals also received 15g of Rat chow a day to make certain nutritional requirements were met (Table 1).

Group	Dose	Food
Experimental	6.5g Maltodextrin	15g Rodent Chow
Control	6.5g Equal (2.5g Aspartame)	15g Rodent Chow

Table 1. Table of food regimen.

Behavioral Testing:

Rats were tested on reference memory task in the radial arm maze for three trials per day for 36 days. The radial arm maze had eight arms (Figure 1).

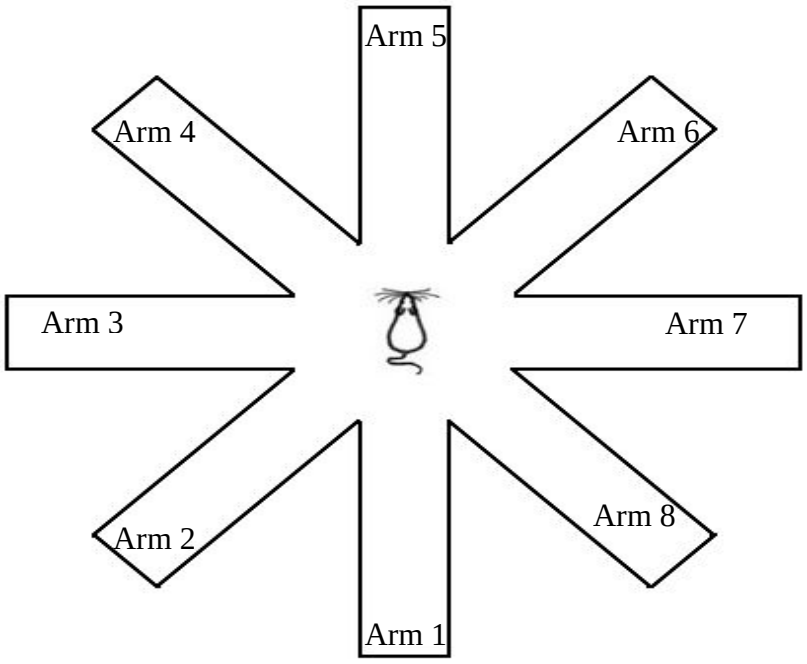

Figure 1. Drawing of radial arm maze

The same arms were baited (1, 2, 5, 7) on each test day using one-quarter of a yellow or orange fruit loop. One trial consisted of the animal being placed in the center of the maze for 10 seconds prior to the start of the trial. Each trial lasted for 3

min. If the animal did not reach all 4 baited arms in 3 min it was removed from the maze. Each animal was also allowed to make a maximum of 10 choices. Once the animal made 10 choices it was also removed from the maze. Finally, if the animal made 4 correct choices, prior to 3 min elapsing or making 10 choices, the animal was removed from the maze. At the end of each trial the rat was removed from the maze and placed back in its home cage. The animal remained there until all animals completed the trial. Animals were returned to their main housing and fed that days food after the third trial.

There were three types of errors that an animal could commit. The first error is called the short-term memory error in which an animal re-enters an arm that was baited. There are then two long-term memory errors called omission and commission. Errors of omission were defined as failing to enter a baited arm prior to 3 min elapsing or making 10 choices. Errors of commission were defined as entering an arm that was never baited.

Extra-maze visual cues consisted of a black sheet with a white triangle on it on one side of the maze, a white wall with a black rectangle, a white curtain, and furnishings in the room at the time of testing such as tables and chairs. These cues are present to serve as visual points of reference to aid in the recall of which arms are baited. One experimenter was present during the time of testing. The experimenter was blind to the condition of the animals.

Histology:

After behavioral testing was completed, the animals were euthanized and perfused for histological analysis. The animals were euthanized with a lethal dose

Nembutal (75 mg/kg, IP) and perfused intracardially with phosphate-buffered saline followed by 10% formalin. The saline solution was pumped throughout the body until the fluid exiting the right atrium became clear. Roughly 500 ml of 10% formalin was then administered throughout the subject's body to preserve the brain tissue. Following this procedure, the animals were then decapitated and the brains were extracted. The brains were then placed in 30% sucrose solution prior to sectioning. Brains were cut with a Leica cm1900 cryostat in 40 micron sections. Sections were stained with cressyl violet and cover slipped for viewing.

Cell counts were then quantified by placing slides on a Ken-A-Vision microprojector. A cell matrix that was 8mm X 8mm with a cross section every .5mm was placed over the medial arcuate nucleus (Figure 2).

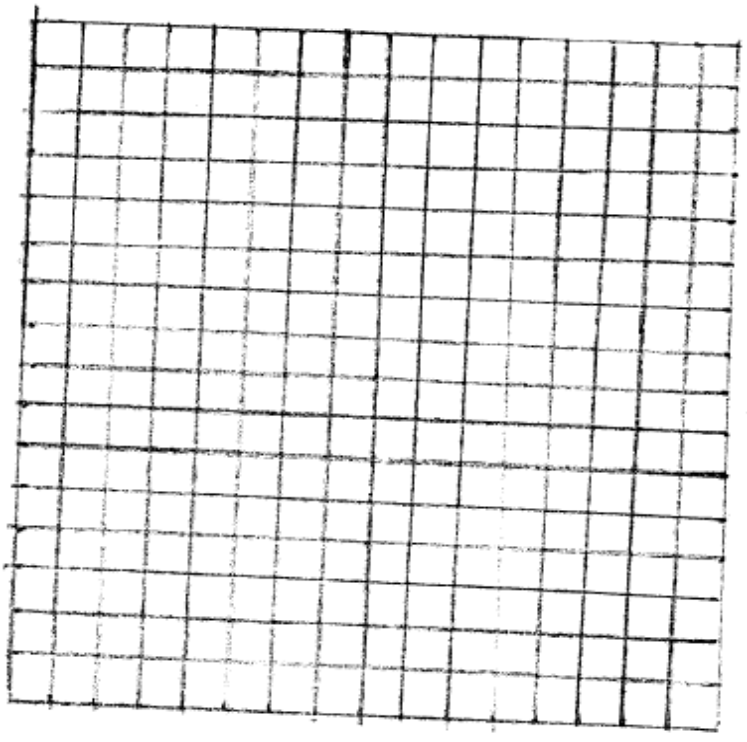

Figure 2. Matrix used to count cells

Cells were only counted if they were in the cross section of the matrix. A maximum of 225 cells could be counted.

Results

Total Errors

A one-way repeated measures analysis of variance (RM-ANOVA) was computed to determine if animals that were given aspartame produced more total errors (omission + commission + short-term errors) in the eight arm radial arm maze. There were two groups in which the average mean and standard error over days was determined: aspartame (M= 2.39, SE= .143) and control (M= 1.49, SE= .138). A significant main effect of days (F (35,875) = 47.412, $p < .001$) was found, in which all animals improved over the span on 36 days. There was no Group X Days interaction (F (35,875) = .879, p = .671). There was a between groups effect in which the control group committed less total errors than the aspartame group (F (1,25) = 20.405, $p < .001$) (Figure 3).

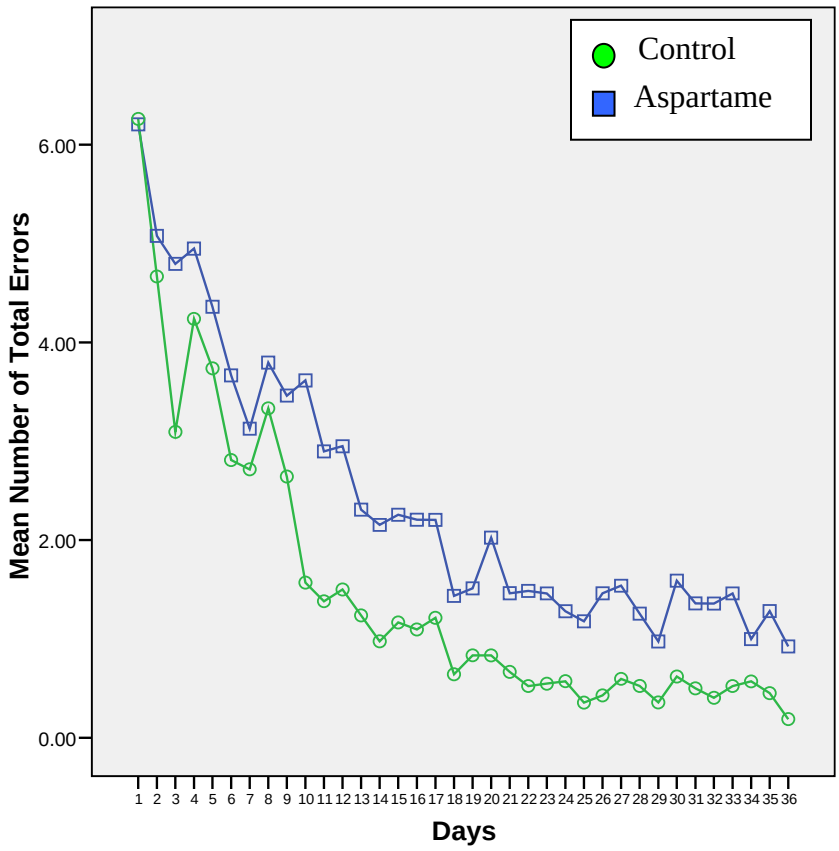

Figure 3. Mean number of total memory errors (omission + commission + short-term errors) across 36 days.

A RM-ANOVA was computed to determine total errors total errors (omission + commission + short-term errors) in the first, second, and third trial across days. In the first trial the two groups mean and standard error over days was: aspartame (M= 3.80, SE= .187) and control (M= 1.05 , SE= .180). On the first trial a significant main effect of days (F (35,875) = 18.629, p < .001) was found, in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 3.521, p < .001). A significant between groups

effect in which the control group committed less total errors than aspartame group in the first trial was also found (F (1,25) = 112.485, p < .001) (Figure 4).

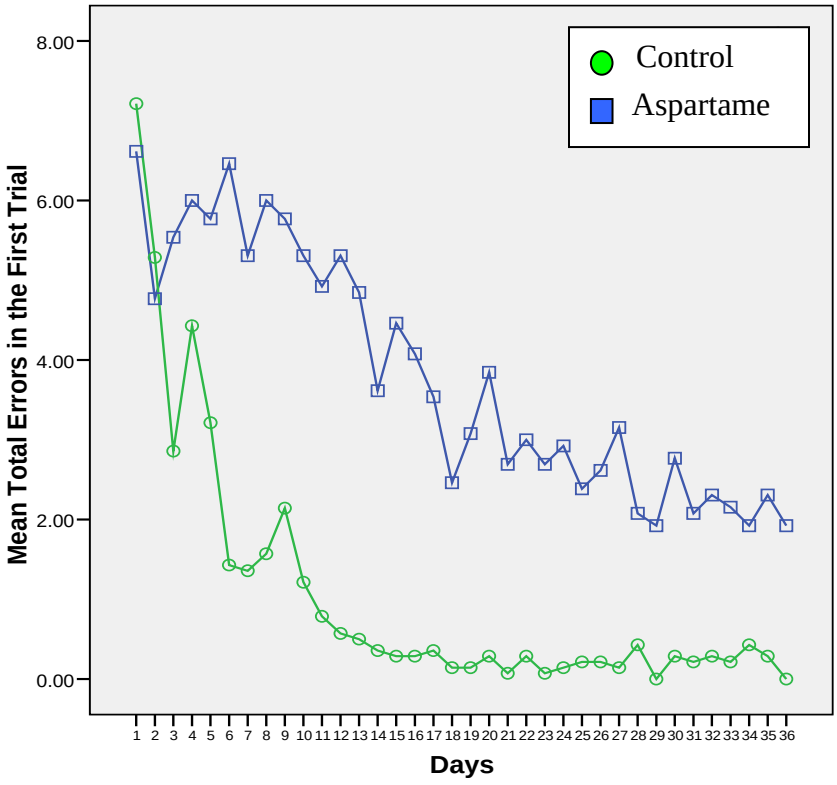

Figure 4. Mean number of total memory errors (omission + commission + short-term errors) across 36 days in the first trial.

The two groups mean and standard error over days on the second trial was: aspartame (M= 1.818, SE= .160) and control (M= 1.833, SE= .155). On the second trial there was a main effect of days (F (35,875) = 27.613, p < .001), in which all animals improved over the span on 36 days. There was also a Group X Days

interaction (F (35,875) = 1.670, p = .009). There was no between groups effects (F (1,25) = .005, p = .947) (Figure 5).

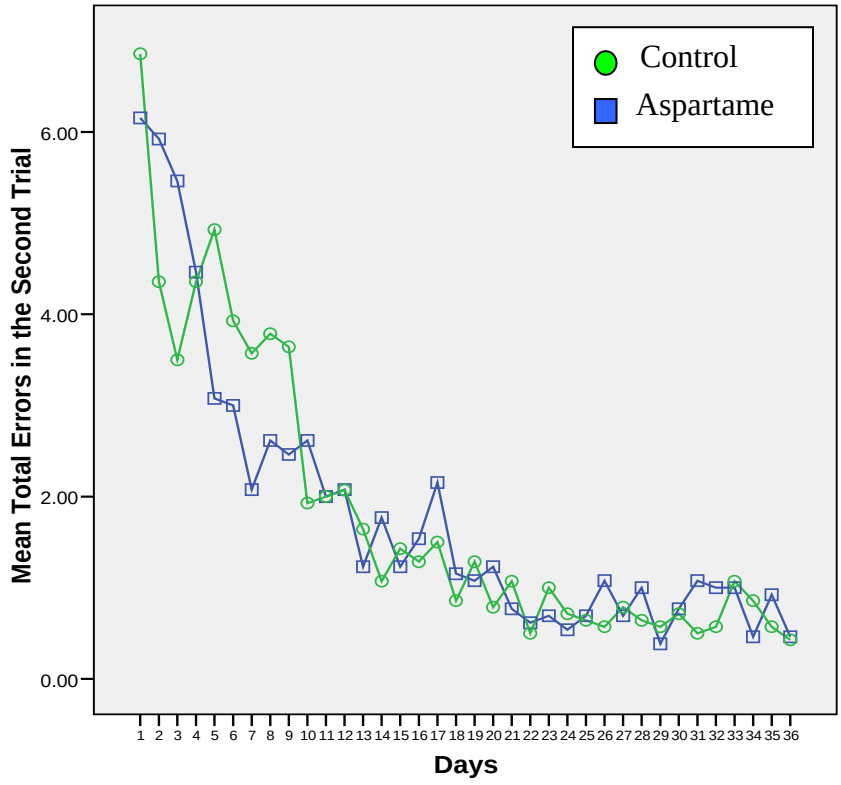

Figure 5. Mean number of total memory errors (omission + commission + short-term errors) across 36 days in the second trial.

Finally, on the third trial the two groups mean and standard error over days was: aspartame (M= 1.56, SE= .123) and control (M= 1.60, SE= .119). On the third trial there was a significant main effect of days (F (35,875) = 22.988, p < .001) was found, in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 1.737, p = .006). There were no between groups effects (F (1,25) = .059, p = .811) (Figure 6).

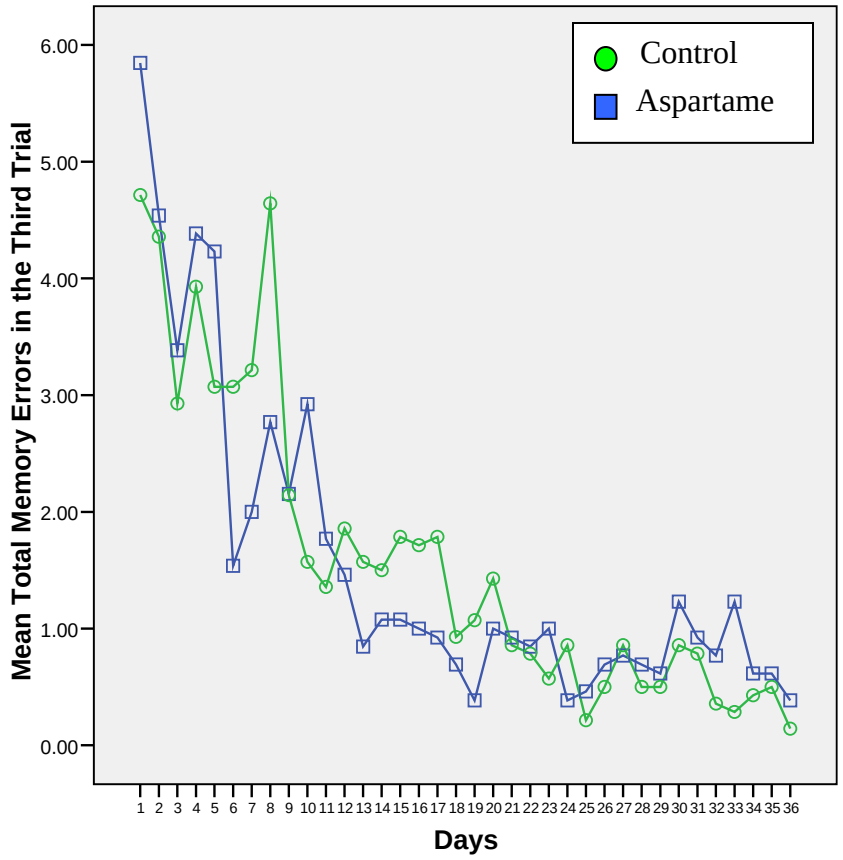

Figure 6. Mean number of total memory errors (omission + commission + short-term errors) across 36 days in the third trial.

Omission + Commission Errors

A RM-ANOVA was computed to determine if animals that were given aspartame produced more total long term memory errors (omission + commission) in the eight arm radial arm maze. There were two groups in which the average mean and standard error was determined: aspartame (M= 1.68, SE= .115) and control (M= 1.03, SE= .111). A significant main effect of days (F (35,875) = 54.448, p < .001) was found, in which all animals improved over the span on 36 days. There

was no Group X Days interaction (F (35,875) = .771, p = .121). There was a between groups effect in which the control group committed less long-term memory errors than aspartame group (F (1,25) = 16.421, p < .001).

A RM-ANOVA was computed to determine total long term memory errors (omission + commission) in the first, second, and third trial across days. In the first trial the two groups mean and standard error over days was: aspartame (M= 2.577, SE= .134) and control (M= .708, SE= .130). In the first trial there was a significant main effect of days (F (35,875) = 19.418, p < .001) was found, in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 2.466, p <.001). A significant between groups effect in which the control group committed less long-term memory errors than aspartame group in the first trial was also found (F (1,25) = 100.130, p < .001). On the second trial the two groups mean and standard error over days was: aspartame (M= 1.31, SE= .122) and control (M= 1.21, SE= .118). In the second trial there was a main effect of days (F (35,875) = 28.954, p < .001), on which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 1.975, p = .001). There was no between groups effects in the second trial (F (1,25) = .290, p = .595). Finally, on the third trial the two groups mean and standard error was: aspartame (M= 1.16, SE= .109) and control (M= 1.18, SE= .105). On the third trial there was a main effect of days (F (35,875) = 23.959, p < .001), in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 1.742, p = .005). There was no between groups effects in the third trial (F (1,25) = .015, p = .903).

Commission Errors

A one-way repeated measures analysis of variance (RM-ANOVA) was computed to determine if animals that were given aspartame produced more commission errors in the eight arm radial arm maze. There were two groups in which the average mean and standard error over days was determined: aspartame (M= 1.55, SE= 1.05) and control (M= .900, SE= .102). A significant main effect for days (F (35,875) = 43.247, p < .001) was found, in which all animals improved over the span on 36 days. There was no Group X Days interaction (F (35,875) = .952, p = .550). There was a between groups effect in which the control group committed less commission errors than the aspartame group (F (1,25) = 19.542, p < .001) (Figure 7).

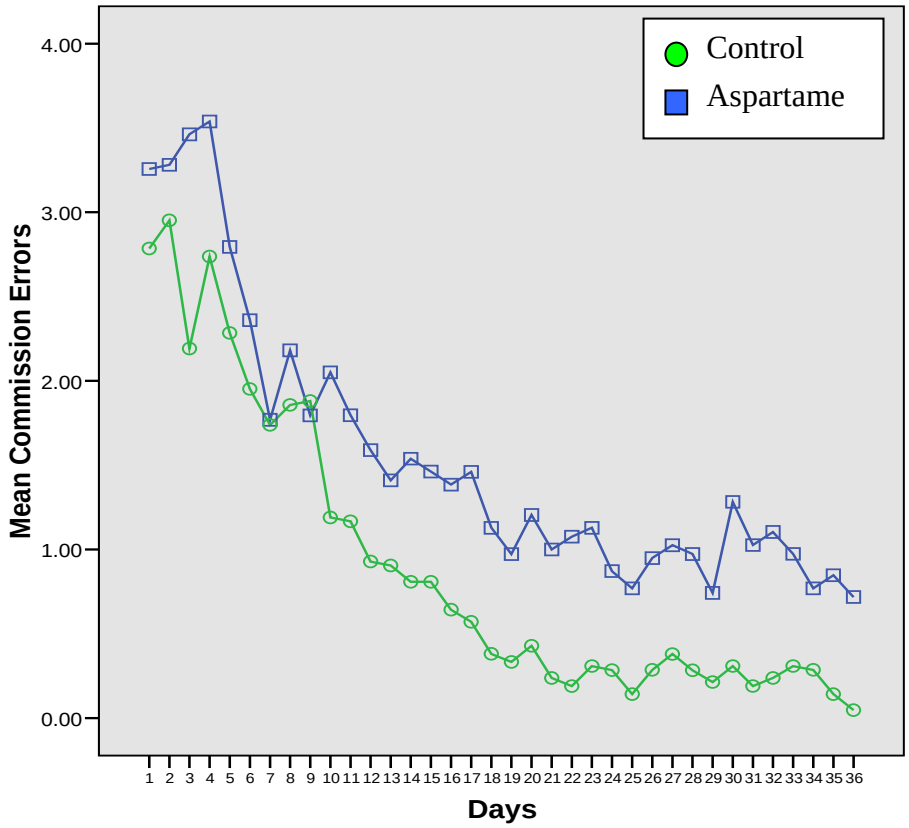

Figure 7. Mean number of commission errors across 36 days

A RM-ANOVA was computed to determine commission errors in the first, second, and third trial across days. In the first trial the two groups mean and standard error over days was: aspartame (*M*= 2.374, *SE*= .120) and control (*M*= .591, *SE*= .116). In the first trial there was a main effect of days (*F* (35,875) = 14.919, *p* < .001), in which all animals improved over the span on 36 days. There was no Group X Days interaction (*F* (35,875) = .952, *p* = .550). A significant between subjects effect in which the control group committed less commission errors than aspartame group in the first trial was also found (*F* (1,25) = 113.852, *p* < .001) (Figure 8).

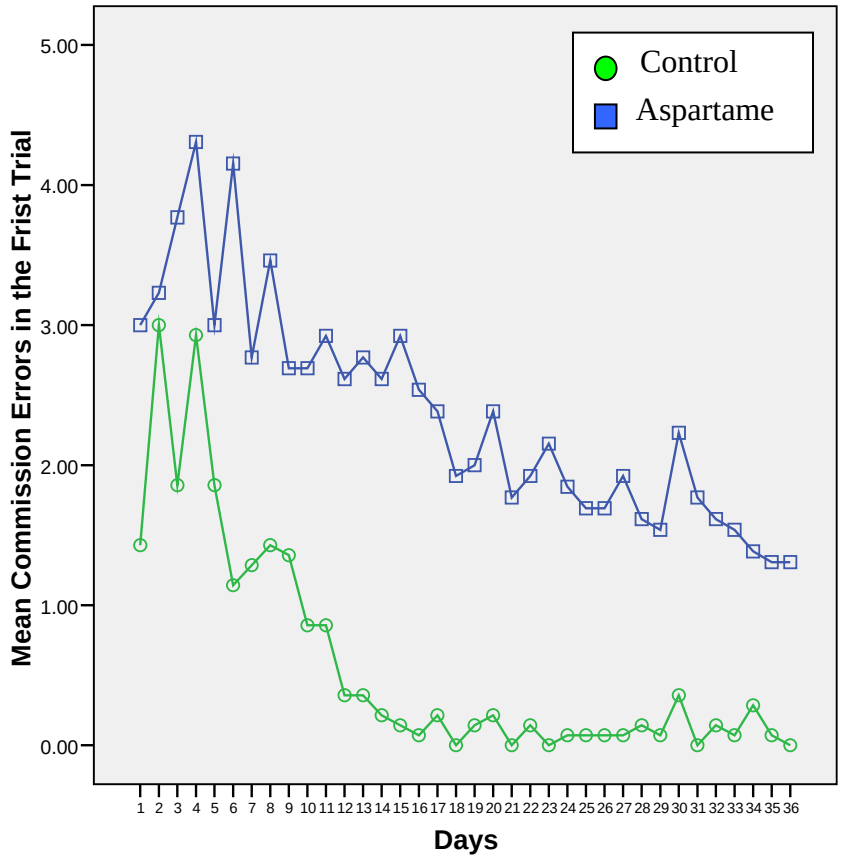

Figure 8. Mean number of commission errors across 36 days in the first trial.

The two groups mean and standard error over days on the second trial was: aspartame (*M*= 1.20, *SE*= .117) and control (*M*= 1.07, *SE*= .113). On the second trial there was a main effect of days (F (35,875) = 23.338, $p < .001$), in which all animals improved over the span on 36 days. The within group interaction of days X group was approaching significance (F (35,875) = 1.39, $p = .066$). There was no between subjects effects in the second trial (F (1,25) = .612, $p = .441$) (Figure 9).

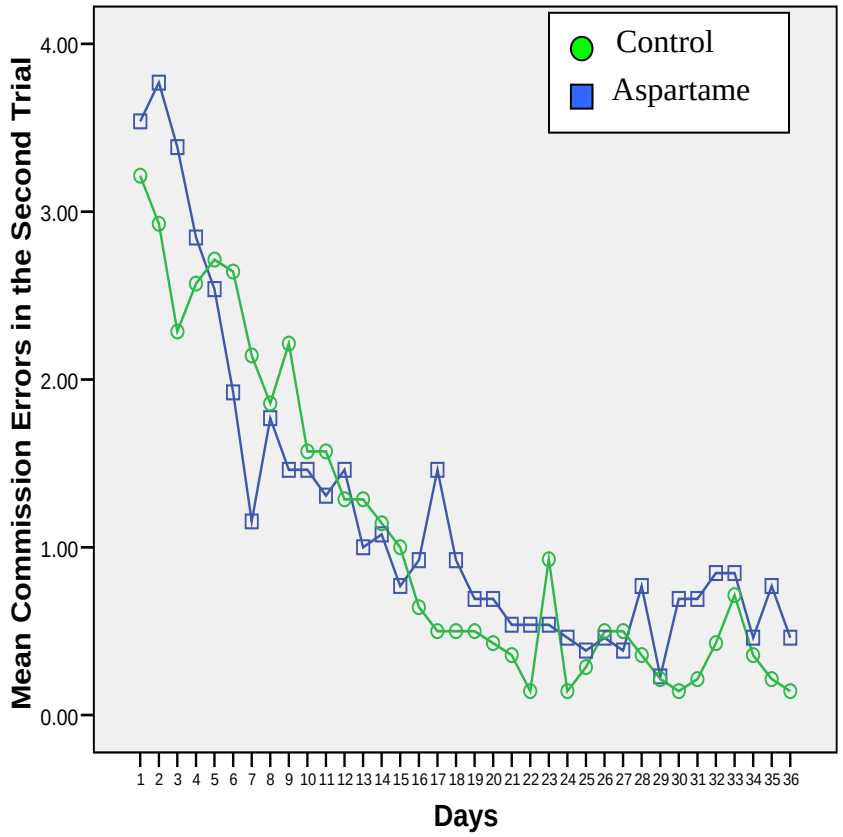

Figure 9. Mean number of commission errors across 36 days in the second trial.

Finally, in the third trial the two groups mean and standard error over days was: aspartame (*M*= 1.066, *SE*= .095) and control (*M*= 1.04, *SE*= .092). In the third trial there was a main effect of days (F (35,875) = 21.765, $p < .001$), in which all animals improved over the span on 36 days. The within group interaction days X group was found to be significant (F (35,875) = 1.70, $p = .007$). There was no between groups effect in the third trial (F (1,25) = .053, $p = .820$) (Figure 10).

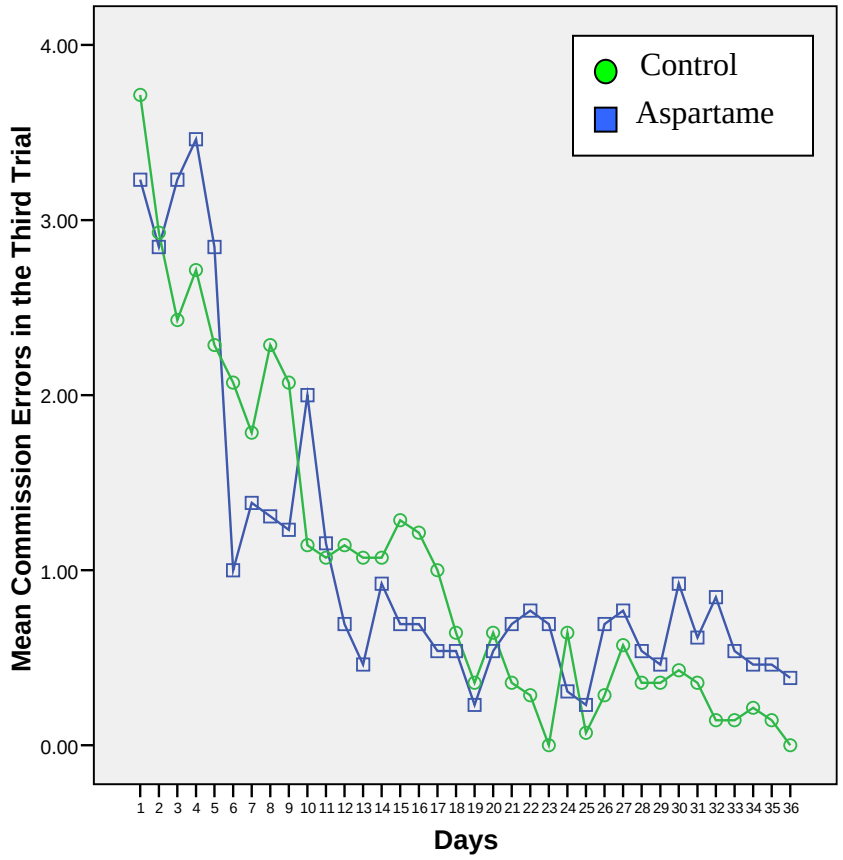

Figure 10. Mean number of commission errors across 36 days in the third trial.

Omission Errors

A RM-ANOVA was computed to determine if animals that were given aspartame produced more omission errors in the eight arm radial arm maze. There were two groups in which the average mean and standard error over days was determined: aspartame (M= .136, SE= .023) and control (M= .117, SE= .022). A significant main effect of days was found (F (35,875) = 28.572, p < .001), in which all animals improved over the span on 36 days. There was a significant Group X

Days interaction (F (35,875) = 1.504, p = .032). There was no between groups effects (F (1,25) = .092, p = .553) (Figure 11).

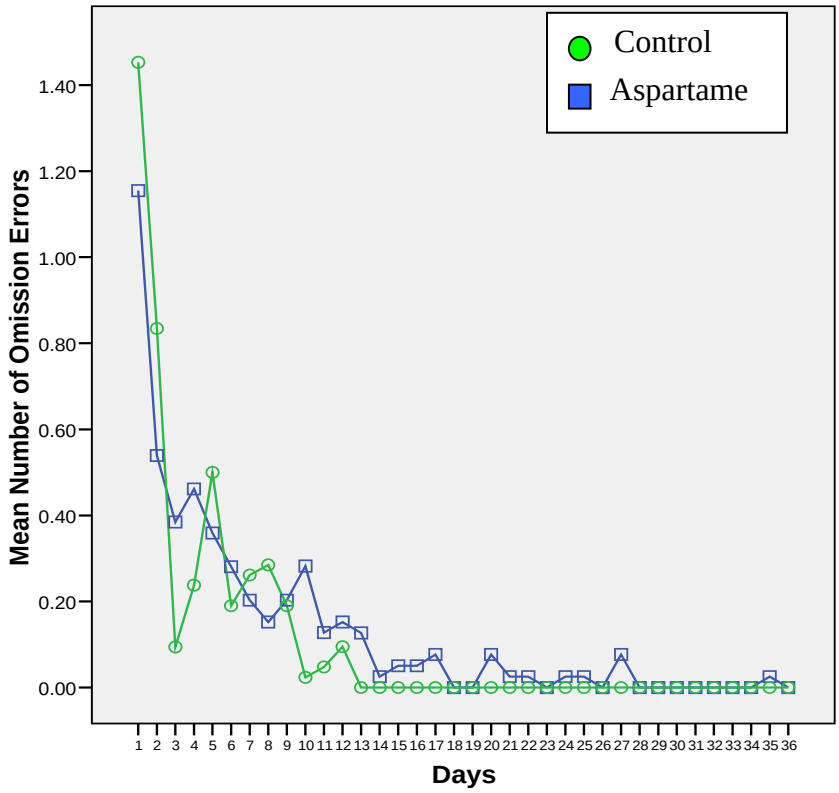

Figure 11. Mean number of omission errors across 36 days.

A RM-ANOVA was computed to determine omission errors in the first, second, and third trial across days. On the first trial the two groups mean and standard error over days was: aspartame (M= .190, SE= .032) and control (M= .153, SE= .031). On the first trial there was a main effect of days (F (35,875) = 19.923, p < .001), in which all animals improved over the span on 36 days. There was also a significant Group X Days interaction (F (35,875) = .298, p < .001). There was not a between groups effect in the first trial (F (1,25) = .718, p = .405) (Figure 12).

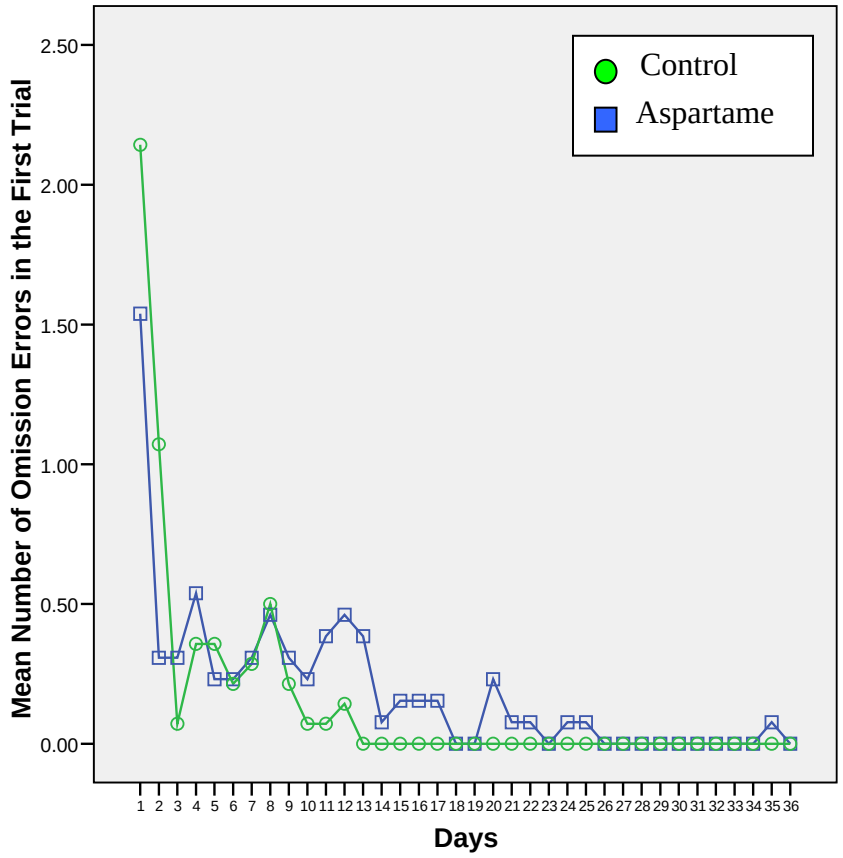

Figure 12. Mean number of omission errors across 36 days in the first trial.

On the second trial the two groups mean and standard error was: aspartame (*M*= 1.28, *SE*= .025) and control (*M*= .095, *SE*= .024). On the second trial there was a main effect of days (F (35,875) = 12.104, $p < .001$), in which all animals improved over the span on 36 days. There was not a Group X Days interaction (F (35,875) = 1.096, $p = .324$). There were no between groups effects in the second trial (F (1,25) = .933, $p = .343$) (Figure 13).

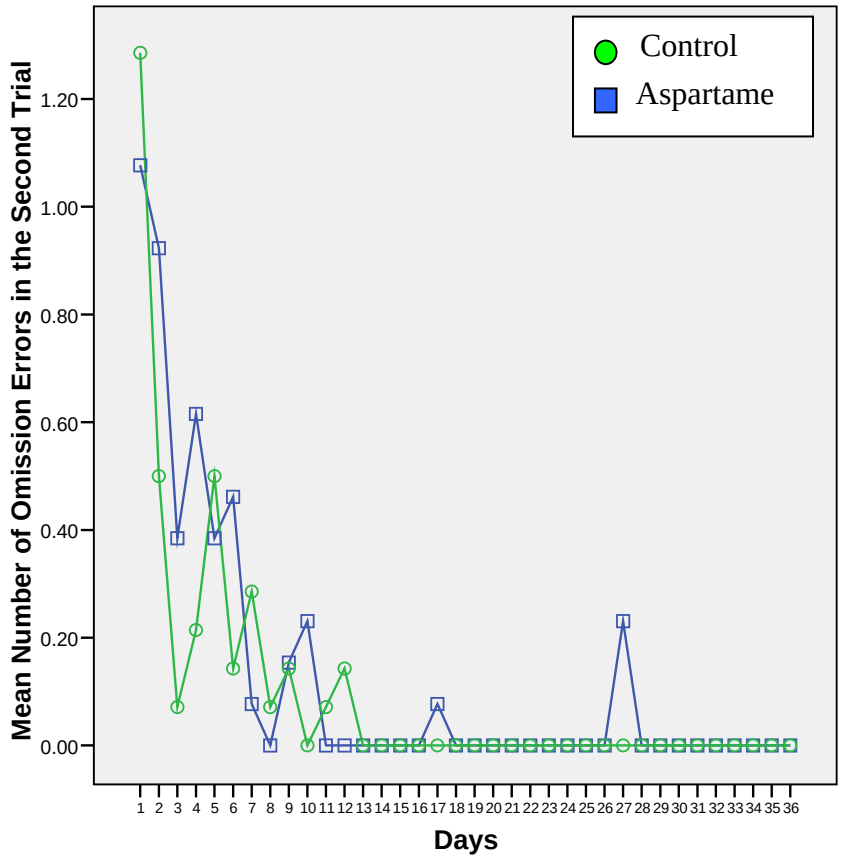

Figure 13. Mean number of omission errors across 36 days in the second trial.

Finally, in the third trial the two groups mean and standard error was: aspartame (*M*= .092, *SE*= .023) and control (*M*= .103, *SE*= .022). On the third trial there was a main effect of days (F (35,875) = 11.523, $p < .001$), in which all animals improved over the span on 36 days. There was not a Group X Days interaction (F (35,875) = .130, $p = .105$). There was no between groups effects in the third trial (F (1,25) = .128, $p = .724$) (Figure 14).

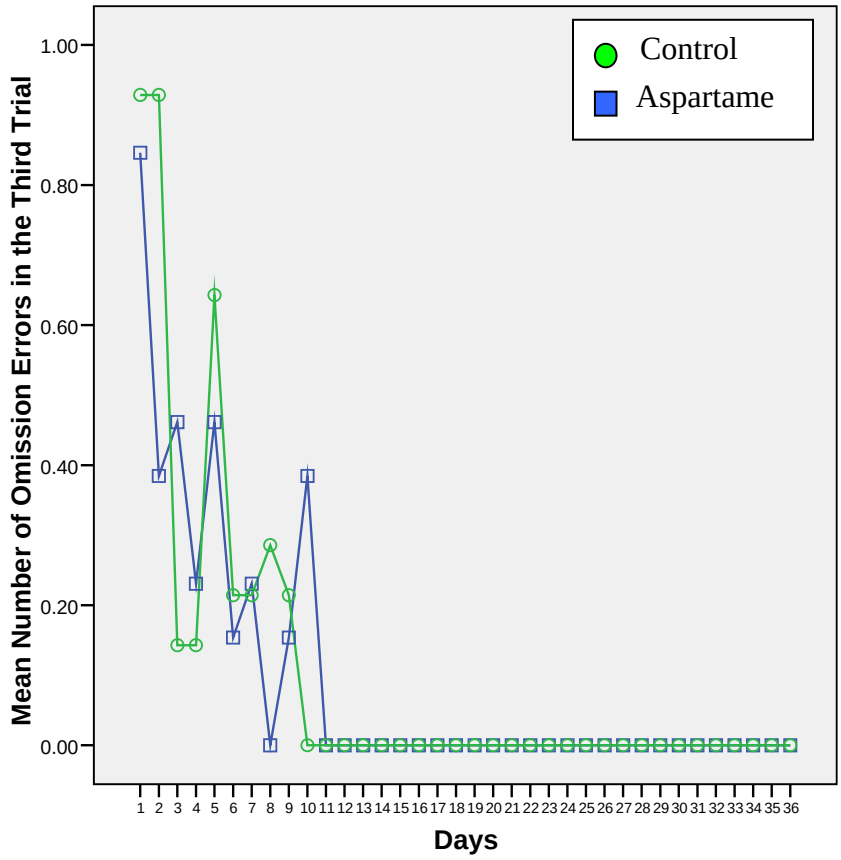

Figure 14. Mean number of omission errors across 36 days in the third trial.

Short-Term Memory Errors

A RM-ANOVA was computed to determine if animals that were given aspartame produced more short-term memory errors in the eight arm radial arm maze. There were two groups in which the average mean and standard error was determined: aspartame (*M*= .720, *SE*= .051) and control (*M*= .500, *SE*= .049). A significant main effect of days (F (35,875) = 11.171, $p < .001$) was found, in which all animals improved over the span on 36 days. There was no Group X Days interaction (F (35,875) = 1.216, $p = .184$). There was a between group effect in

which the control group committed less short-term memory errors than aspartame group (F (1,25) = 9.522, p = .005) (Figure 15).

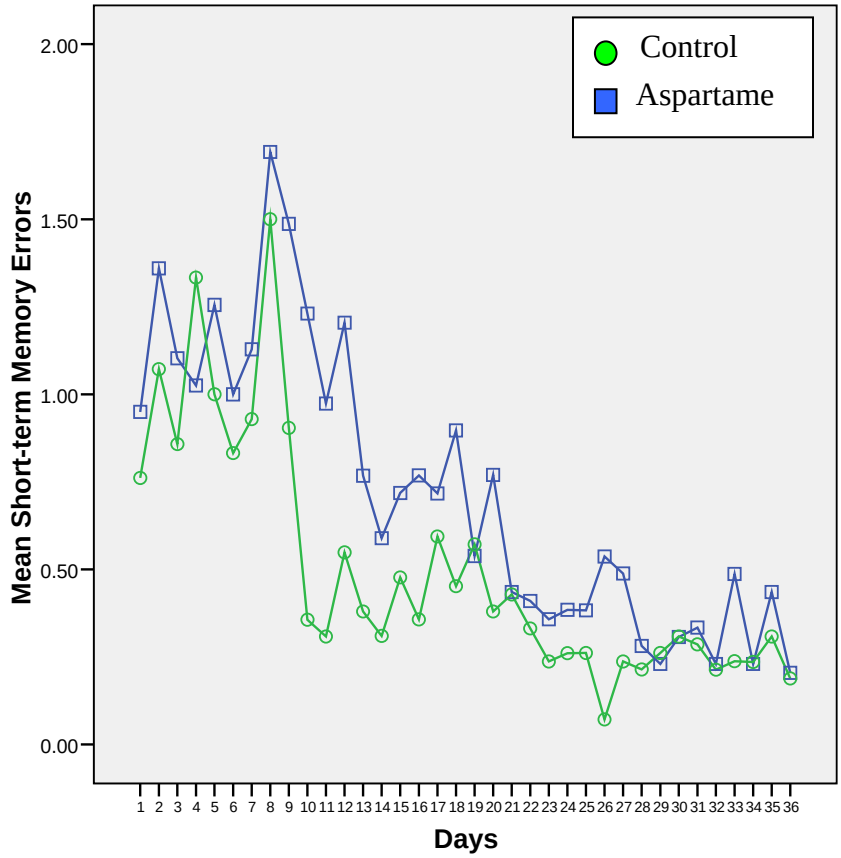

Figure 15. Mean number of short-term memory errors across 36 days.

A RM-ANOVA was computed to determine the short-term memory errors in the first, second, and third trial across days. On the first trial the two groups mean and standard error over days was: aspartame (M= 1.103, SE= .082) and control (M= .323, SE= .079). On the first trial there was a main effect of days (F (35,875) = 4.573, p < .001), in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) = 2.180, p <.001). A

significant between groups effect in which the control group committed less short-term memory errors than aspartame group in the first trial was found (F (1,25) = 46.652, p < .001) (Figure 16).

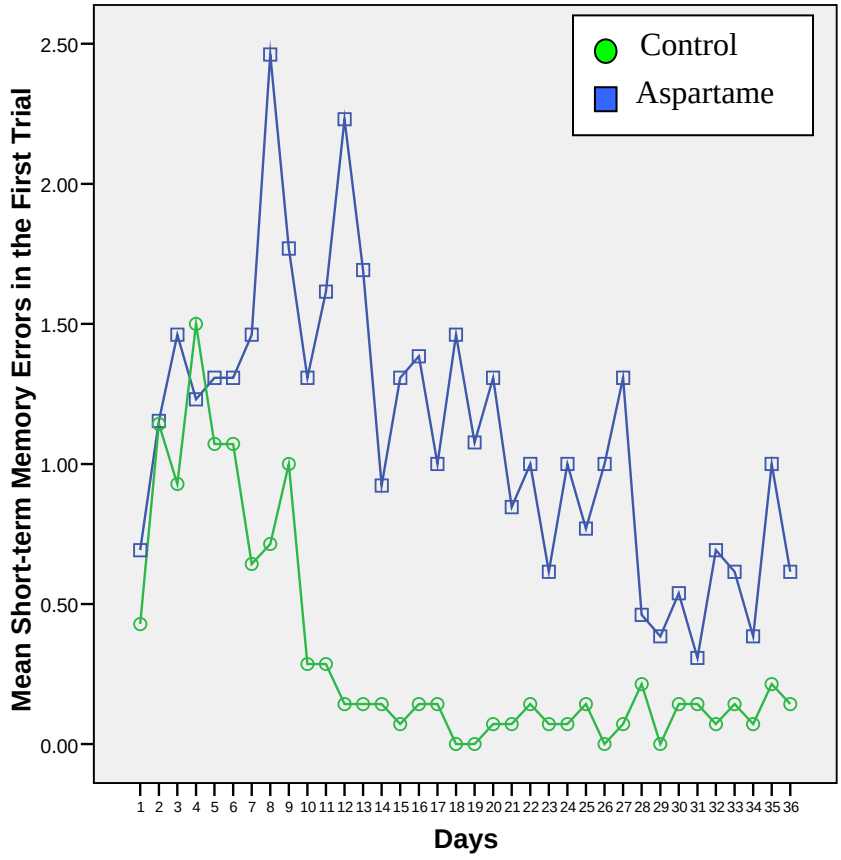

Figure 16. Mean number of short-term memory errors across 36 days in the first trial.

On the second trial the two groups mean and standard error over days was: aspartame (M= .549, SE= .058) and control (M= .617, SE= .056). On the second trial there was a main effect of days (F (35,875) = 5.170, p < .000), in which all animals improved over the span on 36 days. There was also a Group X Days

interaction (F (35,875) = 1.449, p = .046). There was no between groups effects on the second trial (F (1,25) = .711, p = .407) (Figure 17).

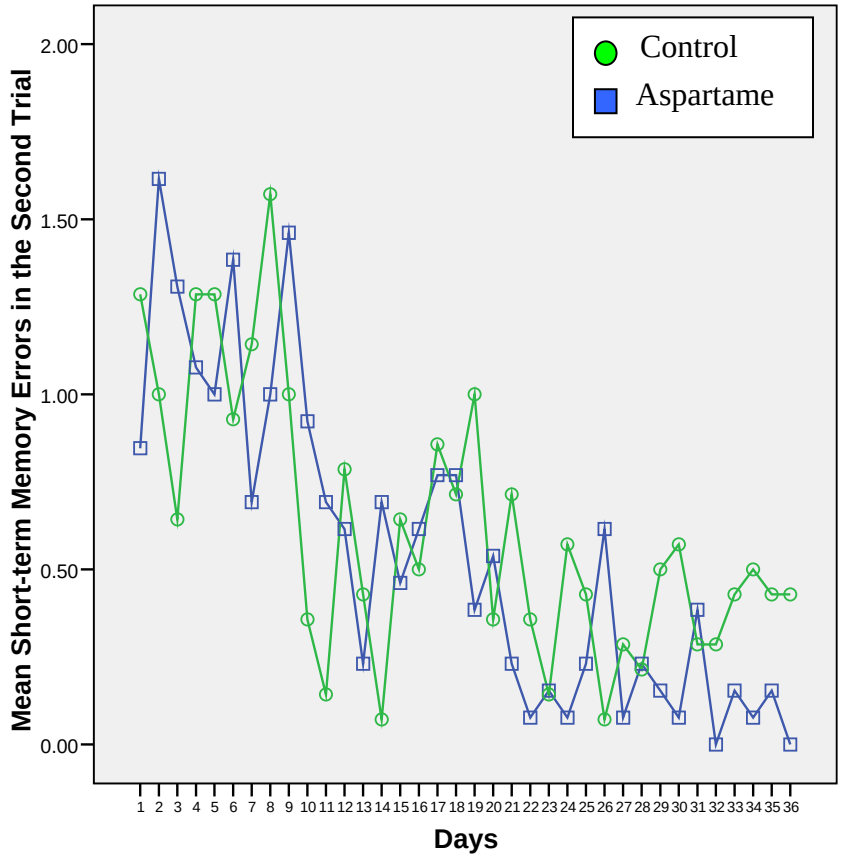

Figure 17. Mean number of short-term memory errors across 36 days in the second trial.

Finally, on the third trial the two groups mean and standard error was: aspartame (M= .509, SE= .041) and control (M= .562, SE= .039). On the third trial there was a main effect of days (F (35,875) = 7.009, p < .000), in which all animals improved over the span on 36 days. There was also a Group X Days interaction (F (35,875) =

1.645, p = .011). There was no between groups effects on the third trial (F (1,25) = .879, p = .358) (Figure 18).

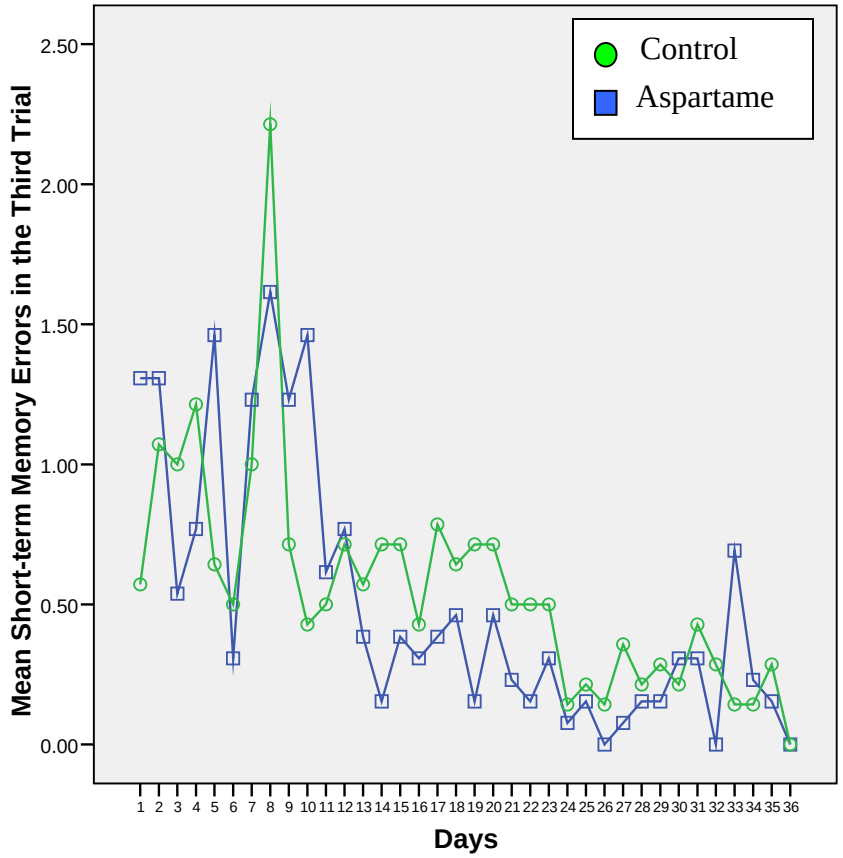

Figure 18. Mean number of short-term memory errors across 36 days in the third trial.

Anatomical Data

Cell counts in the medial arcuate nucleus areas were computed using a 2-tailed t-test. The mean and standard error for the two different groups was computed: aspartame (M= 124.00, SE=3.85) and control (M= 144.43, SE= 3.44).

In the medial arcuate nucleus the control group had significantly more cells than the aspartame group (t (13) = -3.90, p = .002) (Figure 19).

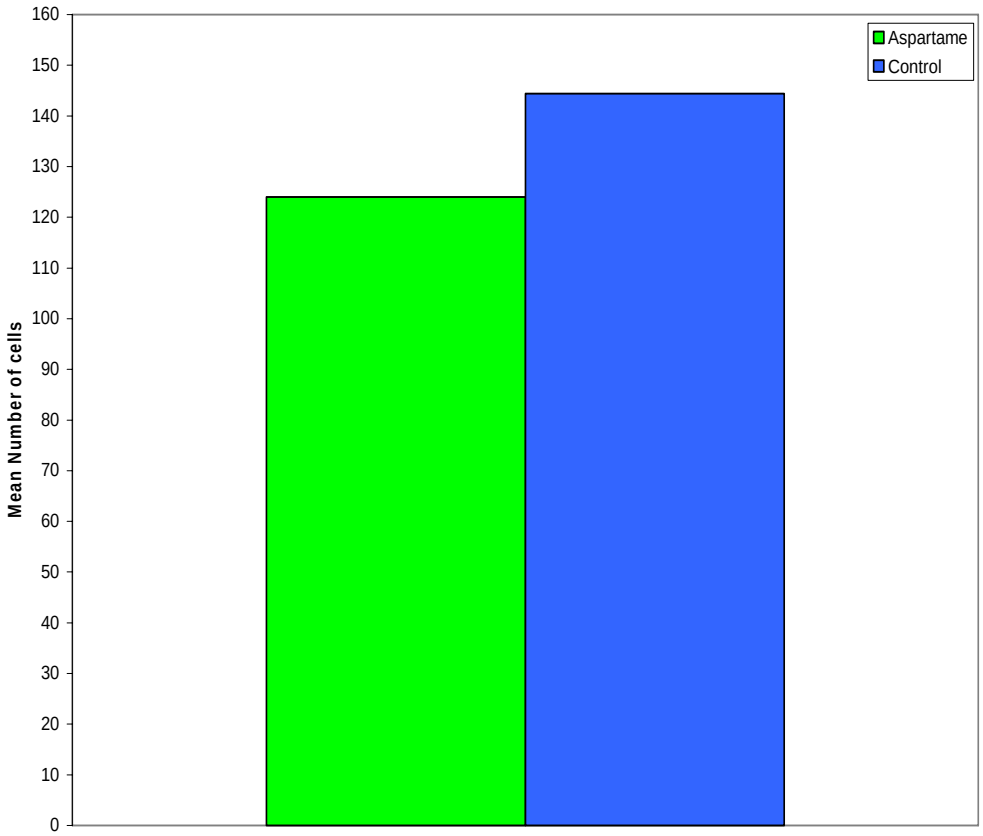

Figure 19. Mean number of cells in the medial arcuate nucleus.

Discussion

The present study investigated whether aspartame influenced memory performance on the eight-arm radial arm maze. Findings revealed that aspartame did have an effect on total errors (Commission + Omission + Short-term errors), long-term memory errors (Commission + Omission errors), and short-term memory errors. These

results support our hypothesis that long-term aspartame consumption leads to a disruption in memory.

Long-term Memory

When omission + commission and commission errors were analyzed there was a significant between groups effect in which the aspartame animals committed more errors than the control group. This group difference is related to performance on the first trial of each day. It seemed to be that the aspartame group "started over" every day in learning the maze. In addition, there were no significant differences between groups on the second or third trials. This may be due to the fact that the aspartame group required more retrieval cues to activate their memories than the control group. There is broad agreement in the literature that retrieval from long-term memory involves some type of parallel search. When a cue is presented, all associated pathways are simultaneously activated and compete in some fashion to retrieve a response (Rickard & Bajic, 2004).

According to Schacter (2001), memory retrieval is a process of accessing stored memories. There are four basic ways in which information can be pulled from long-term memory. The first is recall which involves being able to access information without being cued with any part of the memory. Next, recollection involves reconstructing memory, often utilizing a logical structures, partial memories, narratives or cues. Third, recognition involves identifying information after experiencing it again. Finally, relearning information that has been previously learned would aid in memory retrieval (Schacter, 2001). These four processes need to take place to be able to retrieve memories. One or more of these processes may be disrupted from the use of aspartame.

If this happens it may be an explanation why the memory retrieval process is disrupted following long-term aspartame use.

Neuropsychological evidence has implicated prefrontal cortex in operations engaged during the formation and retrieval of memories for events (Mangels, Gershberg, Shimamura, Knight, 1996). Previous research has shown that left prefrontal cortex is more involved in episodic memory encoding and in semantic memory retrieval, whereas right prefrontal cortex is more involved in episodic retrieval (Tulving, Kapur, Craik, Moscovitch, & Houle, 1994). Research has also suggested that hippocampal formation, together with perirhinal and parahippocampal cortex, participates in explicit memory function. In a study by Buckner and colleagues (1995), found that a region in left lateral prefrontal cortex may be used for the memory retrieval process. In the event that aspartame is damaging any of these areas of the brain it could explain why aspartame may be producing a memory retrieval deficit.

Short-term Memory

When short-term memory errors were analyzed there was a significant between groups effect in which the control animals had less errors than the aspartame group. Upon closer evaluation researchers found that on the first trial of each day the aspartame group exhibited significant deficits in re-entering a baited arm they had previously been in; however, the control group showed continued improvement on consecutive days. In addition, there were no significant differences between groups on the second or third trials. These results suggest that aspartame may disrupt or impair short-term memory. The results of this study are similar to other studies that found an association between aspartame use and short-term memory.

According to Konen and colleagues (2000), college students were surveyed about there past and present aspartame use using the every day memory questionnaire (EMQ) and four short-term memory tasks. Researchers found that three factors emerged: temporal forgetting (problems remembering when to complete a task), forgetting a routine (problems remembering routine tasks), and recoverable forgetting (forgetting a task was competed) (Konen et al., 2000). Animals in the aspartame group may be exhibiting recoverable forgetting. Recoverable forgetting involves forgetting a task was already completed which may be why animals in the aspartame group re-entered a baited arm they had previously been in significantly more than the control group.

Anatomic data

Cell counts in the medial arcuate nucleus areas showed a significant decrease in neurons in the aspartame group compared to the control group. The results of this study are similar to other studies that found an association between aspartame and the degeneration of the arcuate nucleus. According to O1nley and colleagues (1980), when glutamate and aspartate was administered orally it destroyed neurons in the arcuate hypothalamic nucleus. The arcuate nucleus of the hypothalamus is an area particularly vulnerable to glutamate induced damage and has been shown to be completely destroyed following glutamate induced damage (Onley & Ho, 1970). Localization of damage to the arcuate nucleus may be explained in terms of this region having a reduced blood brain barrier. These regions are known as CVO's and that a wide variety of substances having no access in areas with a blood brain barrier readily pass into the CVO. It has been previously shown that aspartate and other structural analogs of

glutamate that mimic the neuroexcitatory action of glutamate also mimic its ability to destroy CVO neurons (Price, Onley, Lowry, & Buchsbaum, 1981). Damage has been shown to be restricted to CVO regions (Price, Onley, Lowry, & Buchsbaum, 1981).

Limitations to the Present Study

Caution must always be taken when generalizing results of nonhuman memory data to humans because the rate of metabolism of these substances varies from species to species, the results are not always comparable. Rats however have a much higher metabolism for both phenylalanine and methanol than humans, and it is difficult to produce toxicity from either of these two components in rats (Fernstrom, Fernstrom, & Gillis, 1983; Stegnik, 1987). Due to these findings if aspartame is producing toxicity in rats and they metabolize it at a faster rate than humans we feel it is safe to generalize these findings to the human population.

Future Directions in Aspartame Research

The present study aimed to reveal whether aspartame influenced memory in the eight arm radial-arm maze. The findings showed that aspartame did have a negative influence on long-term memory and short-term memory. A follow-up study could attempt to determine whether changes to the immune system may exacerbate aspartame induced memory impairments. For example, Lipopolysaccharide (LPS) and aspartame may be administered to healthy adult animals. LPS a non-infectious component of Gram-negative bacterial cell wall induces “sickness behavior” that coincides with the expression and release of proinflammatory cytokines (Sparkman, Kohman, Scott, & Boehm, 2005). Previous research has shown the permeability of the BBB may be altered by LPS (Xaioa, Banks, Niehoff, & Morley, 2001). Aspartame may be able to

cross the BBB at an easier rate and may exacerbate damage to areas that were not penetrated when the BBB was intact. LPS may exacerbate both long-term memory impairments and short-term memory impairments in the radial-arm maze.

Another set of follow up studies could attempt to administer aspartame in a dose dependent manner. The ADI that is currently set in the United States is 50 mg/kg of body weight. In the present study the dose was 250 mg/kg which is considered a high dose of aspartame. A future study should replicate these findings at 250 mg/kg/day and also include and medium and a low dose of aspartame. A dose dependent study could ascertain whether long-term exposure to aspartame may be dangerous at low doses and not just high doses. Aspartame consumption has also increased since the ADI was set and children are consuming 50-77 mg/kg of aspartame a day when given free access to aspartame containing food and beverages which is the ADI set for healthy adults (Pardridge, 1986). According to Mead (2006), the carcinogenic effects are evident at daily doses as low as 400 parts per million, equivalent to an assumed daily human intake of 20 mg/kg body weight. This dosage is much less than the acceptable daily intake for humans, with current limits set at 50 mg/kg in the United States and 40 mg/kg in Europe. 20 mg/kg is within the range of moderately heavy consumers of diet sodas and other artificially sweetened foods. A 140-pound woman would need to drink just three cans of diet soda a day and a 180-pound man would need to drink four cans of diet soda a day (Mead, 2006).

Finally, future studies need to examine other areas of the brain that may be susceptible to aspartame damage. Other areas that are recognized as CVO area that should be looked at include the subfornical organ, area postrema, medial preoptic

nucleus, ventromedial nucleus of the hypothalamus, medial nucleus of the thalamus, and the hypoglossal nucleus (Price, Onley, Lowry, & Buchsbaum, 1981). Due to the memory impairments future studies should quantify cells in the hippocampus (CA1, CA2, CA3, and Dente Gyrus). The formation of new memories and the retrieval of older memories are both evidenced in the hippocampus region of the brain. For this reason future research should look at the hippocampus when examining the effects of aspartame on memory.

The present experiment suggests that aspartame does impair long-term memory and short-term memory. These finding coincide with previous research that has reported memory impairments due to aspartame. According to Christian and colleagues (2004), when rats received aspartame for 3-4 months they took significantly longer to find the reward in the t-maze. Another study found that when aspartame was administered to weanling rats as 9% of the diet (about 11 g/kg/day) for thirteen it altered the learning behavior of male rats (Potts, Bloss & Nutting, 1980). The present experiment also found that cell counts in the medial arcuate nucleus areas showed a significant decrease in neurons in the aspartame group compared to the control group. The results these cell counts are similar to other studies that found an association between aspartame and the degeneration of the arcuate nucleus. With future research the relationship between long-term aspartame use and memory performance will be established and the safety of aspartame will be confirmed.

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Background Information

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ABSTRACT

THE EFFECT OF ASPARTAME ON MEMORY IN RATS

By

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The food additive aspartame, commonly known by its trademark name NutraSweet, is used world wide in over 5000 food and beverages. Anecdotal and experimental evidence suggest aspartame may have detrimental effects on cognition and memory. In the present study, rats were fed aspartame for a 3 month period and their spatial memory was tested in the radial arm maze. It was hypothesized that rats who received aspartame would commit more memory errors than control rats that were not fed aspartame. Previous evidence also suggests aspartic acid creates lesions in the brain areas surrounding circumventricular organs (CVO). The present study attempted to replicate those findings. Results from the present study found that animals that were given aspartame committed significantly more long-term memory errors and short-term memory errors in the eight arm radial-arm maze. The deficits were mostly related to errors in the first trial. The results also suggested animals given aspartame had significantly fewer neurons in the arcuate nucleus when compared to control animals.