

The Effects of Prescribed Nitisinone (Orfadin) on the Anxiety Levels of both FAH<sup>+/+</sup> and FAH

-/- Mice

by

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### **Abstract**

Tyrosinemia Type I (TTI) is an autosomal recessive disorder that is caused by the deficiency of the FAH enzyme, resulting in the elevation of tyrosine and its byproducts in the blood. This build up of toxic metabolites in a child's bloodstream leads to catastrophic effects, eventually leading to death if not treated. However, the only treatment available for children with TTI includes pharmacological measures with Nitisinone (Orfadin) and liver transplantation. Unfortunately, both treatments carry major risks. While liver transplants require life-long management and the risk of rejection, Nitisinone has been linked to severe cognitive impairment while treating children with TTI. With evidence that the only pharmacologic treatment for TTI causes impaired cognition in children, increased awareness and the development of improved treatment is necessary.

A mice-breeding program provided the sample for this study, producing enough FAH +/- (disease carrying) and FAH -/- (diseased) mice to run through mazes testing cognition. The mice were tagged for identification and genotyped at 3-4 weeks old. Thirteen mice were tested through the Elevated Plus Mazes to assess areas of cognition, specifically innate anxiety. This study will analyze the results from the Elevated Plus Maze. Based on the results, diseased mice and disease carrying mice portrayed equal levels of anxiety. Both groups displayed high levels of anxiety when prescribed a low dose of Nitisinone.

Advisor (signature) Date

Department Chair (signature) Date

Honors Program Director (signature) Date

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**Table of Contents**

<b>Abstract.....</b>	<b>2</b>
<b>Acknowledgement .....</b>	<b>3</b>
<b>List of Tables .....</b>	<b>5</b>
<b>List of Figures.....</b>	<b>6</b>
<b>Introduction.....</b>	<b>7</b>
<b>Review of Literature .....</b>	<b>8</b>
<b>Methods.....</b>	<b>12</b>
<b>Results .....</b>	<b>13</b>
<b>Limitations.....</b>	<b>16</b>
<b>Discussion.....</b>	<b>16</b>
<b>References .....</b>	<b>20</b>
<b>Tables .....</b>	<b>21</b>
<b>Figures.....</b>	<b>24</b>

**List of Tables**

<b>Table 1: Elevated Plus Maze Randomization.....</b>	<b>21</b>
<b>Table 2: Statistics for Total Sample in Elevated Plus Maze .....</b>	<b>21</b>
<b>Table 3: Cumulative Distance in Maze for Total Sample .....</b>	<b>22</b>
<b>Table 4: Cumulative Frequency in Each Arm for Total Sample .....</b>	<b>22</b>
<b>Table 5: Number of Excretions during Maze Trial for Total Sample .....</b>	<b>23</b>

**List of Figures**

<b>Figure 1: The Catabolic Pathway of FAH and the Effects of Nitisinone (Orfadin) .....</b>	<b>24</b>
<b>Figure 2: Elevated Plus Maze .....</b>	<b>24</b>
<b>Figure 3: Subject in Elevated Plus Maze .....</b>	<b>25</b>
<b>Figure 4: Total Distance Moved for Total Sample .....</b>	<b>25</b>
<b>Figure 5: Cumulative Duration in Open Arms for Total Sample .....</b>	<b>26</b>
<b>Figure 6: Average Duration in Open Arms of Diseased versus Carrier Sample .....</b>	<b>26</b>
<b>Figure 7: Average Duration in Open Arms of Male versus Diseased Female Sample .....</b>	<b>27</b>
<b>Figure 8: Average Duration in Open Arms for Independent Living versus Dependent Living Sample.....</b>	<b>27</b>

## Introduction

Tyrosinemia is a rare genetic disorder caused by the deficiency in fumarylacetoacetate hydrolase (FAH), an enzyme that breaks down the amino acid tyrosine. As a result, this disease leads to the build up of tyrosine and alpha-fetoprotein in the bloodstream. If untreated, the build up of tyrosine and its byproducts lead to serious medical problems, including hepatic and renal disease (Scott, 2006). There are three types of Tyrosinemia, each with distinctive symptoms but all caused by the elevation of tyrosine: Tyrosinemia type I, Tyrosinemia type II, and Tyrosinemia type III. Of the three types, Tyrosinemia type I (TTI) is the most severe form of the disease (Thimm, Richter-Werkle, & Kamp, 2012).

Tyrosinemia type I (TTI) is an autosomal recessive disorder that can lead to liver failure, kidney failure, neurocognitive decline, and even liver cancer. The only treatments available for TTI include a liver transplant or pharmacotherapy with Nitisinone (Orfadin). Both treatments have great risks. With a liver transplant, the patient will have lifelong immunosuppression and a high risk for rejection. Moreover, liver transplant patients with Tyrosinemia may still spill succinylacetone in the urine with a risk of renal damage and renal failure if not maintained on Nitisinone (Orfadin) post- transplant. TTI can also be treated with Nitisinone and a low-tyrosine diet (Scott, 2006). Nitisinone inhibits 4-hydroxyphenyl-pyruvate dioxygenase, an enzyme in the tyrosine catabolic pathway. By inhibiting the normal catabolism of tyrosine in patients with TTI, Nitisinone prevents the accumulation of catabolic intermediates, which would otherwise be converted to toxic metabolites that are responsible for the observed liver and kidney toxicity seen in these patients (Larochelle & Bussi eres, 2012). However, studies have shown that Nitisinone may lead to neurological deficits. Unfortunately, there are no other pharmacological treatments for children with TTI.

Through the testing of diseased and carrier mice of Tyrosinemia Type I, this study will analyze the neurocognitive deficits associated with Nitisinone in order to determine if these effects are caused by the prescribed medication or by the pathophysiology of the disease itself. Furthermore, this study will determine how severe the neurocognitive effects of pharmacological treatment are for children with TTI and if these effects outweigh the therapeutic measures of the drug. With evidence that Nitisinone causes impaired cognition, increased public awareness and the development of improved treatment is necessary for children with Tyrosinemia Type I.

The research question to be answered in this study is: Are anxiety levels of both FAH  $-/-$  (diseased) mice and FAH  $+/-$  (disease carrying) mice caused by prescribed Nitisinone or by the pathophysiology of the disease itself? The hypothesis of this research states that the levels of anxiety will be increased in diseased mice compared to disease-carrying mice due to the combination of the disease's pathophysiology and the effects of the pharmacological treatment. This study aims to determine the neurocognitive decline associated with pharmacological treatment by testing subjects in the Elevated Plus Maze. The Elevated Plus Maze will analyze neurocognitive behavioral changes expressed by measuring anxiety levels in the mice sample, both FAH  $-/-$  (diseased) mice and FAH  $+/-$  (disease carrying) mice prescribed with Nitisinone.

### **Review of Literature**

Tyrosinemia Type I (TTI) is fatal disease that leads to serious medical problems, including hepatic and renal disease. Currently, the only pharmacological therapy for Tyrosinemia Type I (TTI) is the use of the drug 2-[2-nitro-4-trifluoromethylbenzoyl]-1, 3-cyclohexanedione (NTBC), commonly referred to as Nitisinone (Scott, 2006). Nitisinone prevents the accumulation of tyrosine and toxic metabolites in the bloodstream caused by TTI. By blocking the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme in the tyrosine degradation pathway, Nitisinone can prevent the neurologic crisis, liver failure, and hepatocellular carcinoma

associated with untreated TTI (Laet, Munoz, Jaeken, François, Carton, & Sokal, 2011).

However, this pharmacological therapy is not without major risks. Although Nitisinone treats TTI, the drug ultimately leads to the progression of another type of Tyrosinemia, Tyrosinemia Type III. As shown in Figure 1, Nitisinone biochemically switches the enzymatic defect found in TTI to the defect found in Tyrosinemia Type III by blocking the HPPD enzyme rather than the FAH enzyme. This blockage ultimately leads to elevated tyrosine concentrations. As a result, children with Tyrosinemia Type I on Nitisinone will have impaired cognitive function due to the pharmacological therapy. Although in lower concentrations than found with TTI, the increased levels of tyrosine found in Tyrosinemia Type III is considered to be the main contributing factor in the impaired cognitive functioning of these patients (Thimm, Richter-Werkle, & Kamp, 2012). Interestingly, Nitisinone treatment studies are now finding cognitive deficits in patients with TTI previously only seen in patients with Tyrosinemia Type III (Laet, Munoz, Jaeken, François, Carton, & Sokal, 2011).

However, the neurocognitive impairment associated with Nitisinone treatment is still not proven to be a side effect of the pharmacological treatment rather than the disease pathophysiology itself. Children with TTI untreated for the disease die at an average before the age of two to ten, depending on the phenotypic expression of the disease. As a result, a clear set of clinical manifestations and side effects of the disease are still unknown. Mild impaired cognitive function may be from the pharmacological therapy with Nitisinone or may have been an unobserved clinical manifestation of TTI (Bendadi, Koning, Visser, Hubertus, de Sain, Verhoeven-Duif, Sinnema, van Spronsen, & van Hasselt, 2013).

Bendadi et al. (2013) conducted a longitudinal study to observe the effects of Nitisinone on patients and to determine if these cognitive impairments were a result of the pharmacological

therapy. The study found a significant decrease in intelligence quotient (IQ) in patients with TTI receiving Nitisinone treatment. In fact, an average drop of 27 IQ points was observed over several months in subjects who were regularly tested (Bendadi et al., 2013). Most likely, Nitisinone affects cognitive function indirectly, by inducing profoundly elevated plasma tyrosine levels in patients. The nature of the observed cognitive impairment remains unclear and the pathophysiology causing the impairment is still not fully understood.

Furthermore, neurocognitive impairment present in patients with TTI may be caused not only by pharmacological therapy, but also by a protein-restricted diet. A low protein diet is necessary for children diagnosed with TTI in order to maintain levels of tyrosine below 500  $\mu\text{mol/L}$  in the body and prevent tyrosine crystals from forming in the corneas. The low tyrosine level can only be maintained with a strict diet and the consumption of medical food (Scott, 2006). Tyrosine is an amino acid found in most protein. Due to the deficiency in the FAH enzyme, children with TTI cannot break down the tyrosine obtained through protein. As a result, increased protein in the diet leads to increased levels of tyrosine in the body, resulting in a metabolic crisis (Scott, 2006). A diet low in protein intake is necessary for patients with TTI in order to reduce the risks for a metabolic crisis and neurologic crisis.

According to Bendadi et al. (2013), as levels of tyrosine increase, another amino acid, phenylalanine decreases in the brain because both acids compete for the same transport to cross the blood-brain barrier. As one amino acid increases in blood levels, the other acid will subsequently decrease. This inverse relationship may be a factor for the neurocognitive impairment seen in TTI (Laet, Munoz, Jaeken, François, Carton, & Sokal, 2011). The combination for high tyrosine and low phenylalanine may lead to insufficient phenylalanine transport to the brain, decreasing the amount of phenylalanine available for protein and

neurotransmitter synthesis. Low phenylalanine levels in the brain also affect other amino acids in the brain. Low phenylalanine levels affect serotonin metabolism, causing an inhibition of tryptophan hydroxylase, the rate-limiting enzyme of serotonin synthesis. Behavioral alterations have been observed in humans and mice with a decrease in serotonin levels in the blood (Thimm, Richter-Werkle, & Kamp, 2012). During a research study, Thimm, Richter-Werkle, & Kamp (2012) observed impaired serotonin release in patients with significant tyrosine levels due to TTI and the effects of pharmacological treatment with Nitisinone. Although this research is fairly recent and still being studied, the results could help explain the cognitive impairment in TTI patients.

The disruption of neurotransmitter synthesis, specifically serotonin, caused by the therapeutic treatment of TTI can manifest as cognitive dysfunction and behavioral changes that can be studied using mice and mazes. Specific mazes have been used in studies in order to measure the neurocognitive effects caused by pharmacological treatment and the pathophysiology of specific diseases, such as Tyrosinemia Type I, in patients. These mazes have been utilized to portray the effects of human genetic diseases through mutant mouse models (Sorregotti, Mendes-Gomes, Rico, Rodgers, & Nunes-de-Souza, 2013). For example, the Elevated Plus Maze measures the levels of anxiety observed in test subjects. As shown in Figure 2, the Elevated Plus Maze is designed in the shape of a plus sign, elevated off the ground with beams. The maze consists of two open arms and two closed arms, each with an open roof to hold the subjects in for a certain period of time. Anxiety-like behavior is determined by the percentage of entries and time spent in the open arms versus the closed arms (Sorregotti, Mendes-Gomes, Rico, Rodgers, & Nunes-de-Souza, 2013). Greater time spent in the open arms is indicative of less anxiety because the subject is willing to explore the maze without the security of a closed

wall, as shown in Figure 3. Motor activity can also be evaluated in the Elevated Plus Maze using the absolute number of closed arm entries in the subject (Sorregotti, Mendes-Gomes, Rico, Rodgers, & Nunes-de-Souza, 2013). Using the Elevated Plus Maze can evaluate clinical manifestations and complications of specific diseases and treatments.

### **Methods**

A mice-breeding program provided the sample for this study, producing enough FAH +/- (disease carrying) mice and FAH -/- (diseased) mice to run through the Elevated Plus Maze testing levels of anxiety. After obtaining the sample, the mice were tagged for identification. Each mouse was tagged with an identification number on the outer portion of the ear, allowing for randomization during the trials. At 3-4 weeks old, each subject was genotyped to determine the genetic makeup. Genotyping allowed for differentiating between the FAH +/- (disease carrying) and FAH -/- (diseased) mice. The colony of mice was then segregated according to genotype and placed in a controlled setting for observation in between maze studies. Specific mice needed to be segregated from all other subjects due to their aggressive personality. As a result, some mice lived independently while others lived together in cages to ensure safety of all subjects from the aggressive behavior of specific subjects. The living status of each subject was noted after the trials were completed, as seen in Table 1.

Thirteen mice, both FAH +/- (disease carrying) and FAH -/- (diseased) mice treated with Nitisinone, were tested through the Elevated Plus Maze for neurocognitive impairment, specifically levels of anxiety. Table 1 illustrates the randomization used for the Elevated Plus Maze. The gender, disease status, and living status were noted for each subject in the sample.

Each subject was placed in the center of the Elevated Plus Maze, shown in Figure 2, and left to roam for five minutes. During the trials, movement, speed, and time of each mouse were recorded using the software Ethovision. Ethovision provided data and video recordings of each

subject to be analyzed for results. Results were attained and compared between the FAH +/- (disease carrying) mice and FAH -/- (diseased) mice.

### **Results**

The sample group tested consisted of thirteen mice, seven males and six females. From this sample, nine mice were FAH +/- (disease carrying) mice and four were FAH -/- (diseased) mice. The gender, disease status, and living status were noted for each subject in the sample and presented in Table 1. The mice were between 8-10 weeks of age when tested. Based on the results obtained, FAH +/- (disease carrying) mice and FAH -/- (diseased) had similar levels of anxiety, with little variation. Patterns of anxiety were variable for both FAH +/- (disease carrying) mice FAH -/- (diseased) mice. The data of the Elevated Plus Maze trials were analyzed in different categories to compare and contrast anxiety levels in the sample tested. The categories include 1) cumulative distance in the maze, 2) cumulative frequency in each arm, 3) cumulative duration in open arms, and 4) number of excretions secreted during trial run.

#### **Cumulative Distance in the Maze**

Each mouse was placed in the Elevated Plus Maze for a trial run that lasted five minutes. During the five minutes, the Ethovision software recorded the total distance moved in the arena. As shown in Table 3, the average distance moved for FAH +/- (disease-carrying) mice was 1074.70 cm. The average distance moved for FAH -/- (diseased) mice was 968.78 cm. The FAH +/- (disease-carrying) mice moved an average 105.92 cm farther than the FAH -/- (diseased) mice. Figure 4 also depicts the total distance moved in cm for each mouse in the sample.

#### **Cumulative Frequency in Each Arm**

During the five-minute trial runs in the maze, the Ethovision software recorded the number of times each mouse traveled from one arm of the maze to the other. The four arms of the Elevated Plus Maze are depicted in Figure 2 and Figure 3. The movement from one arm to

another is referred to as frequency. The average frequency for both FAH +/- (disease-carrying) mice and FAH -/- (diseased) mice were analyzed. The average frequency for FAH +/- (disease-carrying) was 10.91 times and the average frequency for FAH -/- (diseased) mice was 12.15 times. The average frequencies in each specific arm for the sample are listed in Table 4. The FAH -/- (diseased) mice moved from one arm to another an average of 1.24 times more than the FAH +/- (disease-carrying) mice.

### **Cumulative Duration in Open Arms**

Through the Ethovision software, the cumulative duration in each zone of the Elevated Plus Maze was recorded for each mouse in the sample. The cumulative duration in the two closed arms, the two open arms, and the center zone of the arena were recorded and listed in Table 2. Most importantly, this study aimed to distinguish the duration of the sample in both *open* arms. Figure 5 depicts the cumulative percentage of each mouse in the open arms of the Elevated Plus Maze. Results were further broken down to compare disease state, gender, and living status of the sample to the cumulative duration in the open arms.

**Average duration in open arms of diseased and disease-carrying sample.** The average duration in the open arms for FAH +/- (disease-carrying) mice was 6.94 seconds and the average duration in the open arms for FAH -/- (diseased) mice was 12.92 seconds. The FAH -/- (diseased) mice spent an average 5.98 seconds longer in the open arms than FAH +/- (disease-carrying) mice. Figure 6 compares the average duration of in the open arms of both the diseased and disease-carrying sample.

**Average duration in open arms of male versus female sample.** The average duration in the open arms for the males in the total sample was 6.73 seconds. The average duration in the open arms for the females in the total sample was 11.18 seconds. Overall, the female sample

spent an average 4.45 seconds longer in the open arms of the Elevated Plus Maze. Results were also recorded for FAH +/- (disease-carrying) males, FAH -/- (diseased) male, FAH +/- (disease-carrying) females, and FAH -/- (diseased) females. The average duration of FAH +/- (disease-carrying) males was 8.41 seconds versus the average duration of FAH +/- (disease-carrying) females, which was 5.57 seconds. FAH +/- (disease-carrying) males had an average of 2.84 seconds longer duration in the open arms of the maze than FAH +/- (disease-carrying) females. The average duration of FAH -/- (diseased) males was 2.60 seconds and the average duration of FAH -/- (diseased) females was 16.80 seconds. FAH -/- (diseased) females had an average of 14.20 seconds longer duration in the open arms of the maze than FAH -/- (diseased) males. Figure 7 compares the average duration of in the open arms of both males and females in the sample.

**Average duration in open arms for independent versus dependent living sample.**

The average duration in the open arms was also differentiated based on the living status of the mice. Some of the mice lived independently in a cage, while the others lived with at least one other mouse. The average duration in the open arms for independent mice was 2.10 seconds and the average duration in the open arms for the dependent mice was 10.79 seconds. The dependent mice spent an average 8.69 seconds longer in the open arms than the independent mice. Figure 8 compares the average duration in the open arms for both independently living and dependently living mice in the sample.

**Number of excretions secreted during trial run.** Throughout each trial, the number of excretions secreted by each mouse was recorded in order to test anxiety levels in the sample. Table 5 shows the total number of feces and urine collected at the end of the five-minute trial for each mouse in the sample. The average number of excretions for FAH +/- (disease-carrying)

mice was 0.94 excretions and the average number of excretions for FAH  $-/-$  (diseased) mice was 1.00 excretions. The FAH  $-/-$  (diseased) mice had an average of 0.06 excretions more than the FAH  $+/-$  (disease-carrying) mice.

### **Limitations**

Aspects of this research study limited the results obtained. These limitations include sample size, accessibility to the pharmacological agent Nitisinone, and time frame of the study. With only thirteen mice in the sample, there was not enough data to obtain conclusive results comparing the FAH  $+/-$  (disease-carrying) mice and FAH  $-/-$  (diseased) mice when on prescribed pharmacological therapy. There were only four FAH  $-/-$  (diseased) mice in the sample analyzed for this research compared to the nine FAH  $+/-$  (disease-carrying) mice. A larger sample of diseased and carrier mice would have provided an opportunity to analyze more trends in the data. Furthermore, Nitisinone was difficult to access throughout the timeframe of this research. Only small amounts of the drug were accessible and at low doses. The drug was also very expensive to resupply. Outside factors also limited the results obtained in the trials. During the specific trials, background noise at times would distract the mice. As a result, it was not clear if the mice stayed in specific zones of the maze due to anxiety or distraction from the noise. The time frame was also limited in this trial. Obtaining the sample, breeding, genotyping, and running the mazes was all completed within a four-month time frame. More time would have allowed more breeding and a larger sample for the research study.

### **Discussion**

This study used quantitative data to analyze the neurocognitive effects of prescribed Nitisinone treatment on patients with Tyrosinemia Type I. By conducting timed trials on mice subjects, this study analyzed the anxiety-like behavior in FAH  $+/-$  (disease-carrying) mice and

FAH  $-/-$  (diseased) mice in the Elevated Plus Maze. The Ethovision software recorded each trial, measuring the movement, speed, and time of each subject tested in the maze. The results obtained through this research are aimed to portray the neurocognitive effects found in children with TTI prescribed pharmacological therapy with Nitisinone.

The hypothesis of this study states that the levels of anxiety will be increased in FAH  $-/-$  (diseased) mice compared to FAH  $+/-$  (disease-carrying) mice due to the combination of the disease's pathophysiology and the effects of the pharmacological treatment. However based on the results, the levels of anxiety for both diseased and disease-carrying mice were at similar levels, with no obvious distinction between the two groups. Levels of anxiety were tested using several measure in the Elevated Plus Maze. These measure include 1) cumulative distance in the maze, 2) cumulative frequency in each arm, 3) cumulative duration in open arms, and 4) number of excretions secreted during trail run. The analysis of each result allowed for measurement of the subject's level of anxiety.

According to the results for cumulative distance moved in the sample, FAH  $+/-$  (disease-carrying) mice moved an average 105.92 cm farther than the FAH  $-/-$  (diseased) mice. Decreased movement in the diseased mice shows that they were less anxious to explore the unfamiliar environment of the Elevated Plus Maze. Unlike the disease-carrying mice, the diseased were less likely to move around. However, the distance moved in both groups was less than expected. These results support the hypothesis stating that Nitisinone treatment affects neurocognitive function, both in diseased and disease-carrying subjects. Further testing needs to be done in the future to determine if these results are consistent.

Levels of anxiety in the sample were also measured based on the frequency of the subject going from one arm of the maze to another. Based on the trials, the FAH  $-/-$  (diseased) mice

moved from one arm to another an average of 1.24 times more than the FAH +/- (disease-carrying) mice. These results are not as expected according to the hypothesis of this research. Diseased mice were expected to stay in one area of the maze, too anxious to move from one arm to another. However, the FAH -/- (diseased) mice moved more frequently than the FAH +/- (disease-carrying) mice. Although the diseased mice did move more frequently, the diseased sample still did not move as far in distance as the disease-carrying sample. This data is important and may indicate that FAH -/- (diseased) mice moved around more frequently but stayed in the same zone of the maze rather than venturing out to different arms. More testing and analysis needs to be conducted to understand why the diseased mice were more frequent in movement yet lower in total distance moved.

Another measurement of anxiety was the average duration of the mice in the open arms of the Elevated Plus Maze. A longer duration in the open arms is indicative of less anxiety because the test subject is bold enough to venture out where there are no closed walls for protection. The FAH -/- (diseased) mice spent an average 5.98 seconds longer in the open arms than FAH +/- (disease-carrying) mice, an unexpected finding due to the diseased sample's lower total distance moved. However, one female diseased mouse, identity #18, started the trial on the open arm and did not move positions for over eighteen seconds. Since only four results were obtained for FAH -/- (diseased) mice in this study, this result may have skewed the final data and increased the average percentage of FAH -/- (diseased) mice in the open arms. Average durations in the open arms were also compared for independent living subjects versus dependent living subjects and males versus females. These results were obtained for observation and for future data if needed.

The last measure calculated the number of excretions secreted during the maze trial for

each mouse. The FAH  $-/-$  (diseased) mice had an average of 0.06 excretions more than the FAH  $+/-$  (disease-carrying) mice. However, there is no definitive research on the relationship between excretions during a trial and the levels of anxiety in the mice tested. The observation of increased excretions in FAH  $-/-$  (diseased) mice is notable and may indicate an increased level of anxiety of the mice as they ventured through the unknown maze.

All these measures provide data to analyze the levels of anxiety in the FAH  $-/-$  (diseased) mice and FAH  $+/-$  (disease-carrying) mice. The results analyzed thus far have shown no significant difference in the anxiety levels of both groups. However, both diseased and disease-carrying mice showed high levels of anxiety. A future goal of this study is to compare both the FAH  $-/-$  (diseased) mice and FAH  $+/-$  (disease-carrying) mice to wild-type (non diseased) mice in order to determine if the neurocognitive effects witnessed are caused by the pathophysiology of the disease itself, the pharmacological treatment with Nitisinone, or a combination of both. With the results analyzed so far, neurocognitive effects are seen in both FAH  $-/-$  (diseased) mice and FAH  $+/-$  (disease-carrying) mice. However, the difference in the neurocognitive effects, specifically on the levels of anxiety, still needs to be further examined.

All data obtained in this study is designed to help improve the care of children with Tyrosinemia Type I treated with Nitisinone. This study analyzed the results from the Elevated Plus Maze. Based on the results, diseased mice and disease carrying mice portrayed equal levels of anxiety. Both groups displayed high levels of anxiety when prescribed a low dose of Nitisinone. Further research is ongoing and the research team will continue to breed the colony and perform multiple maze studies with the goal of enhanced understanding of the disease and the neurocognitive changes seen in patients on Nitisinone. The team is committed to the long-

term goals of improved patient outcomes and investigation of improved therapies for children with TT1.

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## Tables

Table 1: Elevated Plus Maze Randomization

Identity	FAH	Sex	Color	Cage
17	WT (+/-)	M	brown	individual
33	WT (+/-)	M	brown	(33, 34, 36)
46	D (-/-)	M	light brown	(43, 44, 46)
26	WT (+/-)	M	black	individual
43	WT (+/-)	M	brown	(43, 44, 46)
34	WT (+/-)	M	brown	(33, 34, 36)
44	WT (+/-)	M	brown	(43, 44, 46)
18	D (-/-)	F	white	(18, 20, 21)
42	WT (+/-)	F	light brown	(38, 42, 45, 47)
20	D (-/-)	F	white	(18, 20, 21)
32	WT (+/-)	F	brown	individual
38	D (-/-)	F	white	(38, 42, 45, 47)
21	WT (+/-)	F	brown	(18, 20, 21)

**TOTALS:**

13 total mice tested – 7 males, 6 females

9 WT (Wild Type Carriers)– 6 males, 3 females

4 D (Diseased) – 1 male, 3 females

Table 2: Cumulative Duration in Each Zone of Elevated Plus Maze for Total Sample

Identity	Total Arena	Center Zone	Closed Arm 1/ Center	Closed Arm 2/ Center	Open Arm 1/ Center	Open Arm 2/ Center
	seconds	seconds	seconds	seconds	seconds	seconds
#17	299	63.50	88.10	141.70	1.20	4.50
#33	303.8	21.30	140.00	139.10	2.60	0.80
#46	300.6	20.50	48.40	229.10	2.60	0.00
#26	304.4	32.00	112.40	154.50	0.40	5.10
#43	302.3	89.20	114.50	59.10	26.10	13.40
#34	301.1	22.60	119.20	159.30	0.00	0.00
#44	300.1	132.00	35.30	95.30	37.10	0.40
#18	300.4	88.60	61.30	86.40	51.60	12.50
#42	299	43.00	80.30	162.60	9.10	4.00
#20	301.1	165.60	18.00	106.70	1.40	9.40
#32	303.7	13.60	288.70	0.00	0.80	0.60
#38	300.3	33.90	78.50	162.00	23.60	2.30
#21	300.8	42.30	67.60	172.00	15.00	3.90
Average duration in open arm for carrier mice: 12.92						
Average duration in open arm for diseased mice: 6.94						
*Red indicative of diseased mouse						

**Table 3: Cumulative Distance in Maze for Total Sample**

<b>Total Distance Moved for All Sample</b>	
<b>Identity</b>	<b>cm</b>
#17	1179.04
#33	1341.08
#46	913.96
#26	1025.84
#43	1040.80
#34	1034.00
#44	1003.72
#18	1082.84
#42	1273.01
#20	625.59
#32	1004.11
#38	1252.64
#21	770.72
Average distance for carrier mice: 1074.70	
Average distance for diseased mice: 968.76	
*Red indicative of diseased mouse	

**Table 4: Cumulative Frequency in Each Arm for Total Sample**

<b>Identity</b>	<b>Center Zone</b>	<b>Closed Arm 1 / Center</b>	<b>Closed Arm 2 / Center</b>	<b>Open Arm 1 / Center</b>	<b>Open Arm 2 / Center</b>
#17	32	11	17	2	2
#33	27	10	14	2	2
#46	21	4	16	3	0
#26	18	5	9	1	6
#43	56	27	13	12	10
#34	0	5	13	0	0
#44	24	7	11	5	2
#18	47	4	8	18	18
#42	33	7	20	9	3
#20	25	4	7	3	11
#32	8	6	0	2	1
#38	27	5	14	5	3
#21	28	1	16	11	3
Average frequency for carrier mice: 10.91					
Average frequency for diseased mice: 12.15					
*Red indicative of diseased mouse					

**Table 5: Number of Excretions during Maze Trial for Total Sample**

<b>Identity</b>	<b>Feces</b>	<b>Urine</b>
#17	4	0
#33	0	0
#46	2	0
#26	3	1
#43	3	0
#34	0	0
#44	0	0
#18	1	0
#42	3	0
#20	1	1
#32	0	0
#38	3	0
#21	3	0
Average excretions for carrier mice: 0.94 Average excretions for diseased mice: 1.00 *Red indicative of diseased mouse		

## Figures

Figure 1: The Catabolic Pathway of FAH and the Effects of Nitisinone (Orfadin)

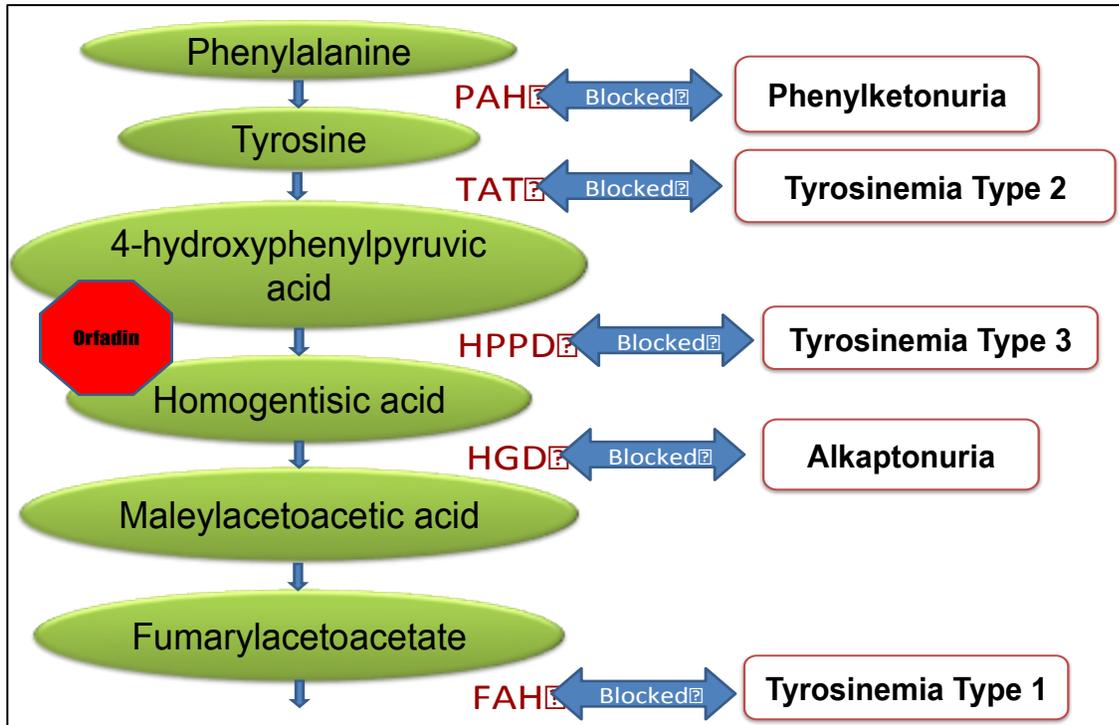
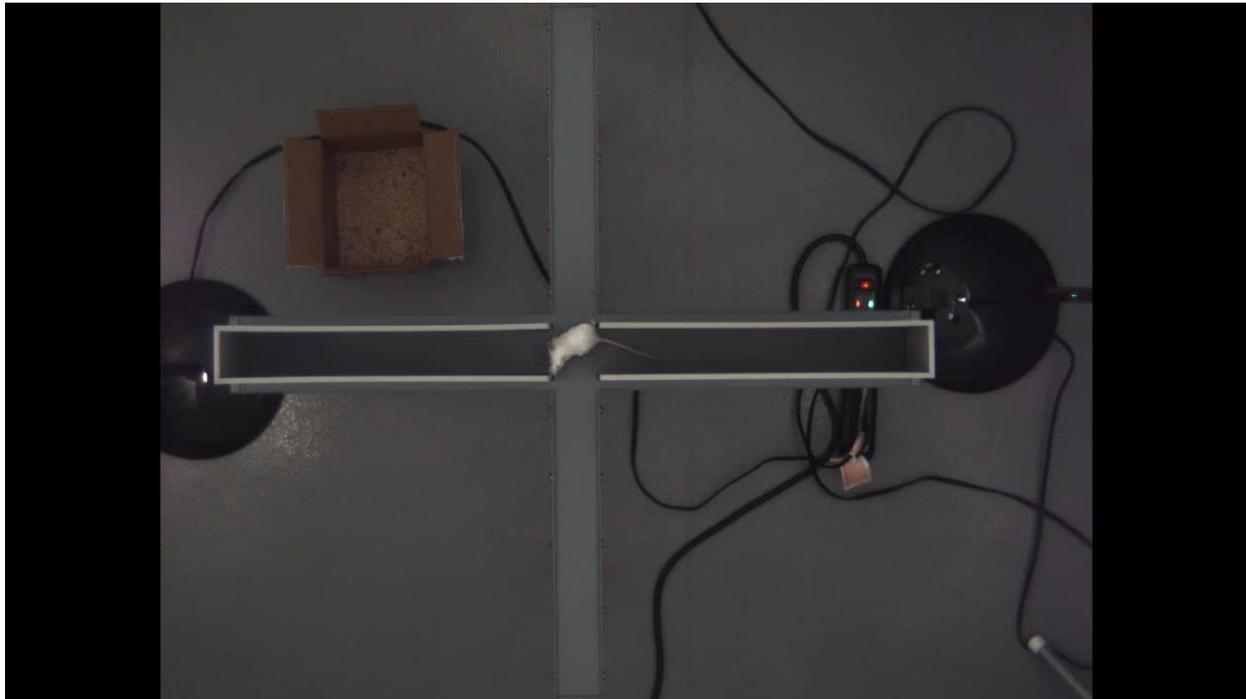


Figure 2: Elevated Plus Maze



Overview of Elevated Plus Maze with open arms and closed arms, surrounded by a white curtain barrier to decrease outside stimulant on the sample

**Figure 3: Subject in Elevated Plus Maze**



Subject exploring center of the Elevated Plus Maze, in between two open arms and two closed arms

**Figure 4: Distance Moved for Total Sample**

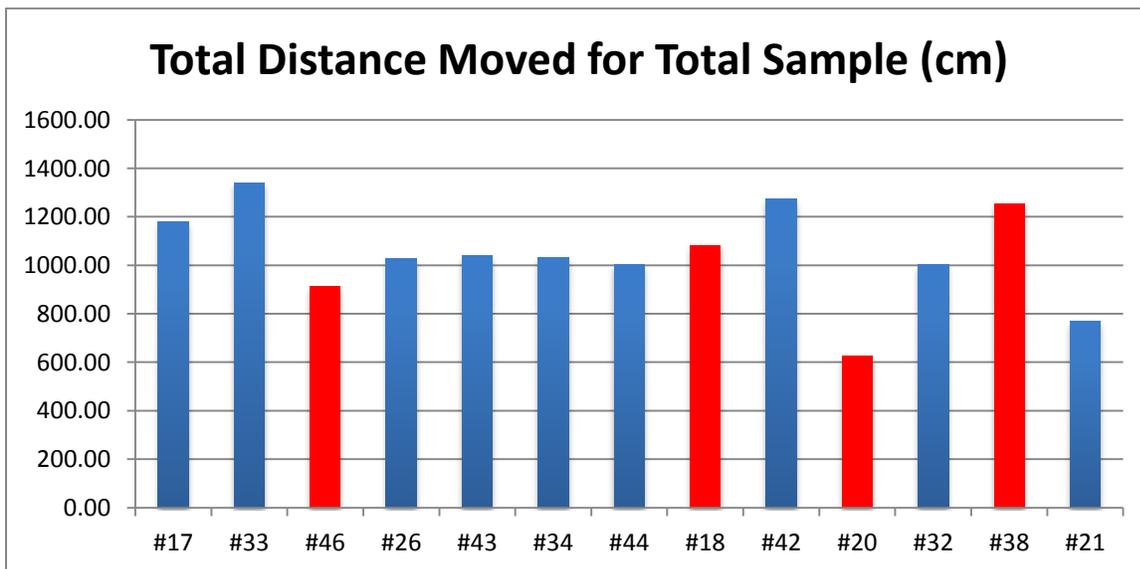


Figure 5: Cumulative Duration in Open Arms for Total Sample

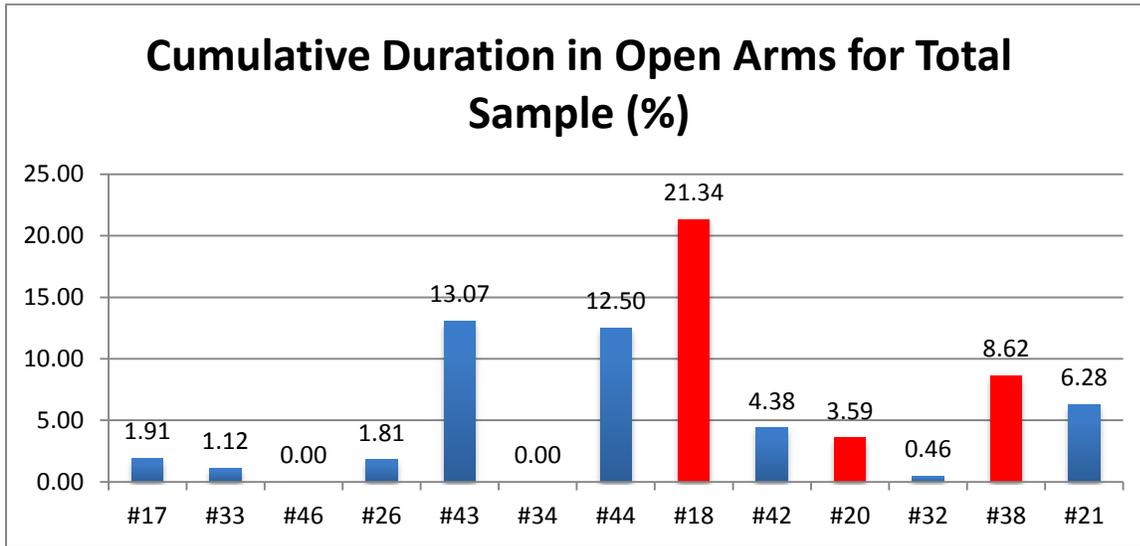
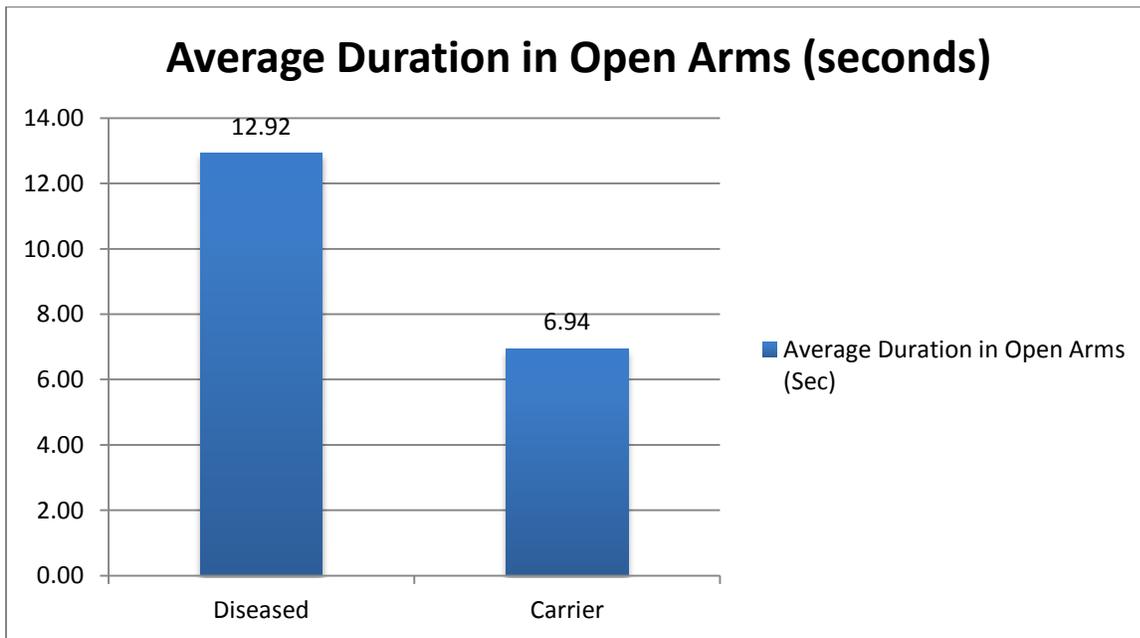
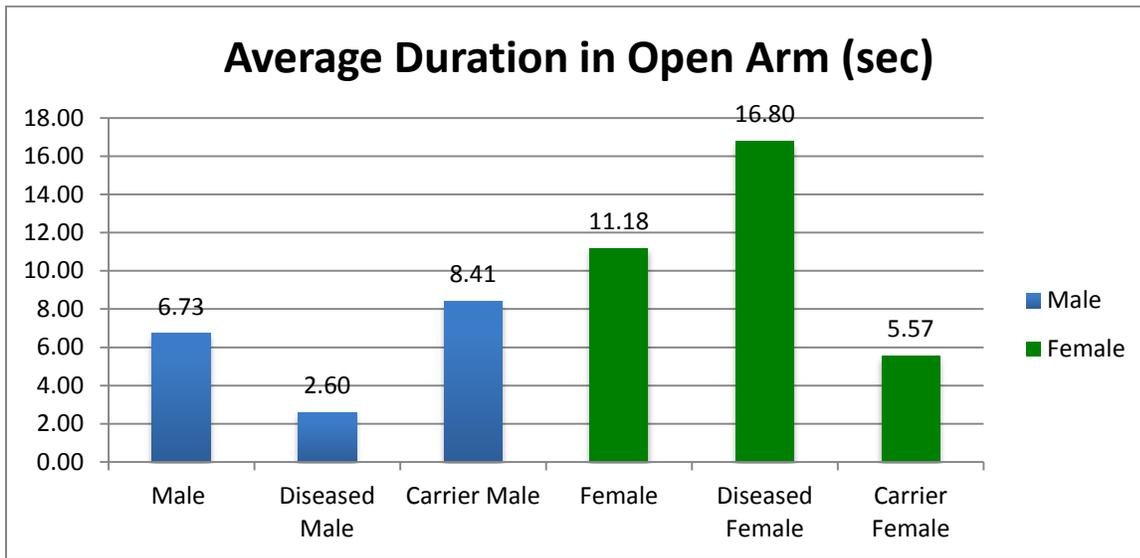


Figure 6: Average Duration in Open Arms of Diseased versus Carrier Sample



**Figure 7: Average Duration in Open Arms of Male versus Diseased Female Sample**



**Figure 8: Average Duration in Open Arms for Independent Living versus Dependent Living Sample**

