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The Analysis of Progranulin Mutations as a Causative Agent in Frontotemporal Dementia

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By

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Emily Lumley

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APPROVED:

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Zuoshang Xu, Ph.D.
Biochemistry and Molecular Pharmacology
UMass Medical Center
Major Advisor

____

David S. Adams, Ph.D.
Biology and Biotechnology
WPI project Advisor

ABSTRACT

Recently, it has been discovered that null mutations in progranulin is a cause of familial frontotemporal dementia. To understand the role of progranulin in this neurodegenerative disorder, transgenic null mice and *in vitro* knockdown of mRNA for progranulin were analyzed. After verifying the transgenic mice using Polymerase Chain Reaction to verify progranulin knockdown, the HomeCageScan System and the rotarod test were performed to investigate motor neuron function. *In vitro* progranulin knockdown in NSC-34 cells was confirmed using RNA extraction and northern blots. The cells that exhibited at least 50% knockdown will be further analyzed in the morphological changes associated with the progranulin haploinsufficiency.

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BACKGROUND

Familial Frontotemporal Dementia

Definition

Frontotemporal dementia (FTD) is a disorder that involves the neurodegeneration of the frontal and temporal lobes (Rosso, et al., 2001). The typical onset of FTD ranges from 40 to 64 years of age, with an average duration of between two to ten years (Mayo Clinic Staff, 2007). It is the second most common cause of presenile dementia, following Alzheimer's disease. FTD accounts for up to 20% of all dementia beginning before the age of 65 years. (Snowden, Neary, & Mann, 2002).

Symptoms

The signs and symptoms of FTD vary from person to person, however the most common abnormalities can be categorized as behavioral changes, speech and language comprehension, and movement disorders. Unlike Alzheimer's disease, memory problems are not associated with FTD in the early stages (Mayo Clinic Staff, 2007).

The most common presentation is changes in behavior and personality. These are extreme changes which include lack of judgment and inhibition, decline in personal hygiene, apathy, increasingly inappropriate actions, and repetitive compulsive behavior (Mayo Clinic Staff, 2007).

Changes in language function are a less common symptom of FTD. Difficulties that may develop consist of problems in the expression of language, reading, and writing. As the disease

progresses, mutism may ensue and language is used less and less (The Regents of the University of California, 2008).

Movement disorders similar to Parkinson's disease can occur in a subtype of FTD called corticobasal degeneration. The signs and symptoms include poor coordination, rigidity, impaired balance, tremor, and muscle spasms. A loss in the ability to carry out purposeful movements can also occur. Frontotemporal dementia with motor neuron disease (FTD/MND) is when movement problems occur along with symptoms of amyotrophic lateral sclerosis. Weakness, muscle shrinkage, muscle spasms, and difficulty swallowing are common signs (Mayo Clinic Staff, 2007).

Causes

FTD has a strong genetic component. The disorder is 35-50% familial with autosomal dominant inheritance. In 1998, the first mutation that was identified as being linked to FTD was found on chromosome 17 for microtubule-associated protein tau (MAPT) (Mackenzie, et al., 2006). Since then, over thirty nine different MAPT mutations have been discovered. These mutations are characterized by tau-positive inclusions and account for 10-20% of familial FTD (Eriksen & Mackenzie, 2008).

However, there have been cases of familial FTD with linkage to the same chromosome 17q21 but lack MAPT mutations (Baker et al., 2006; Eriksen & Mackenzie, 2008). These cases demonstrated tau-negative, ubiquitin-positive inclusions (Goedert & Spillantini, 2006), which represents highly-regulated protein degradation through the ubiquitin protease pathway (Boston Biochem, Inc., 2005). Recent discoveries have identified the cause of these tau-negative FTD cases as mutations in the progranulin gene on chromosome 17q21. With 5-10% of FTD patients

having mutations in the progranulin gene, it is as common as MAPT mutations (Eriksen & Mackenzie, 2008). Figure 1 below shows the location of the progranulin and MAPT gene on chromosome 17q21.

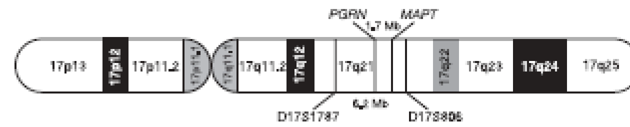


Figure 1: Chromosome 17 with MAPT and progranulin locations marked. Mutations in both these regions on chromosome 17q21 cause FTD. The schematic representation provided by (Baker, et al., 2006).

Progranulin

Location and Function

Progranulin is glycoprotein consisting of 593 amino acids whose gene is found on chromosome 17q21.32. It is present in a variety of tissues throughout the body, including in epithelial and hematopoietic cells (Eriksen & Mackenzie, 2008). Progranulin is multifunctional secreted growth factor involved in cell cycle progression and cell motility. The function of progranulin also includes development, wound repair, and inflammation (Mackenzie, et al., 2006).

In the central nervous system, the role of progranulin is unsure, however, it is extensively expressed during early stages of neural development. Later, progranulin is only expressed in certain neuronal populations such as cortical and hippocampal pyramidal neurons and purkinje cells. In activated microglial cells, progranulin expression is up-regulated (Van Damme, et al., 2008).

Role in FTD

Previous studies have shown FTD is a result of mutations of the progranulin gene, including frameshift, splice-site, nonsense, signal peptide, Kozak sequence disruption, and missense mutations. The majority of these mutations result in premature termination codons which leads to the degradation of the mutant mRNA by nonsense mediated decay. The outcome is a functional null allele, or non-functional or unstable proteins in the case of a missense mutation (Eriksen & Mackenzie, 2008).

Unlike tauopathies, which share the pathological feature of accumulation of insoluble hyperphosphorylated tau proteins, the mutant progranulin is absent in the cells of the nervous system of FTD patients. Instead, the disorder is a result of a loss of functional progranulin. The progranulin gene consists of several different regions, and through past studies, it was concluded that mutations in the various regions result in FTD through the common mechanism of haploinsufficiency (Mackenzie, et al., 2006).

The effects of the deficiency of functional progranulin can be studied through a knockdown approach. RNA interference can be used to mimic the disease in order to observe and analyze the impact of this missing protein.

RNA Interference

Application

RNA interference (RNAi) is tool used in a variety of applications to down-regulate the expression of certain genes for basic studies of gene function or translational research. The uses of RNAi in translational research include producing models for human diseases, exploring the possible side effects of prospective drugs, and testing potential gene therapies (Wiznerowicz, Szulc, & Trono, 2006).

RNAi Systems

The desirable product of RNAi is knockdown that can be externally controlled. There are two systems that result in different types of knockdown. Inducible knockdown usually involves the use of chemicals and is used to research the function of genes that are necessary for development into differentiated tissues in transgenic models. Conditional knockdown is commonly used to produce human pathologies in vivo, which allows for the elimination of disease-influencing genes to be induced and reverted at will. This type of knockdown can be either reversible or nonreversible (Wiznerowicz, Szulc, & Trono, 2006).

Reversible conditional systems involve pharmacologically managed expression of specific interfering RNAs. For the research on progranulin, micro-RNA (miRNA) was used as the interfering RNA for *in vitro* experiments. When doxycycline is added, the miRNA is induced, which complements the target RNA in order to prevent the translation of a specific protein. Doxycycline-controlled units are commonly used since it is easy to add to cell cultures or distribute to animals (Wiznerowicz, Szulc, & Trono, 2006).

Figure 2 shows the design and mode of action of a reversible system through the mechanism of steric hindrance. This model uses doxycycline as the inducer. In the absence of the inducer, the interfering RNA, in this case the silencing RNA (siRNA), is not expressed. This is because the tetracycline repressor (tetR) is blocking the promoter. As soon as doxycycline is added to the system, the tetR is sequestered, and the transcription of the siRNA is induced.

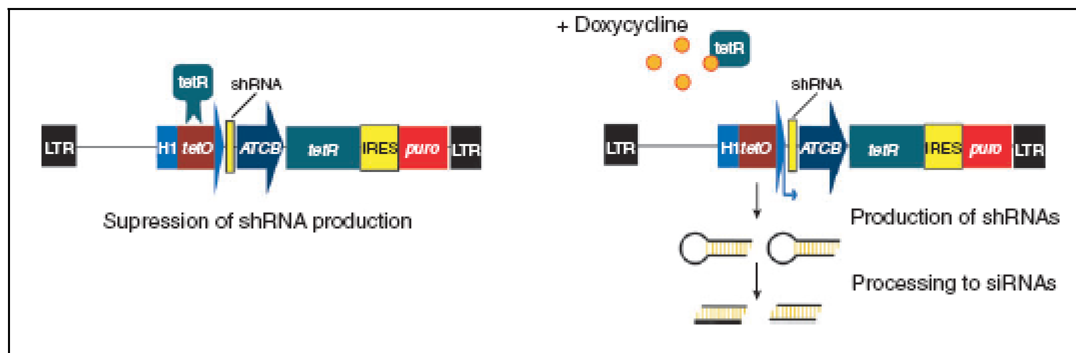


Figure 2: Reversible conditional system involving steric hindrance-mediated repression before and after doxycycline is added; provided by (Wiznerowicz, Szulc, & Trono, 2006).

Irreversible conditional systems produce permanent knockdown. It involves recombinases such as Cre or Flp to excise an inactivating sequence which then induces the expression of the interfering RNA. This is a one-time event that cannot be switched back into the original state. The irreversible systems are generally used to create conditional-knockdown animals or models (Wiznerowicz, Szulc, & Trono, 2006).

Figure 3 illustrates the design of the Cre-inducible miRNA complex. Without the addition of Cre, the Enhanced Green Fluorescent Protein (EGFP) is expressed under green fluorescent light. Once Cre is added, the fragment between the loxP is cut out, allowing the Red Fluorescent Protein (RFP) and miRNA to be expressed.

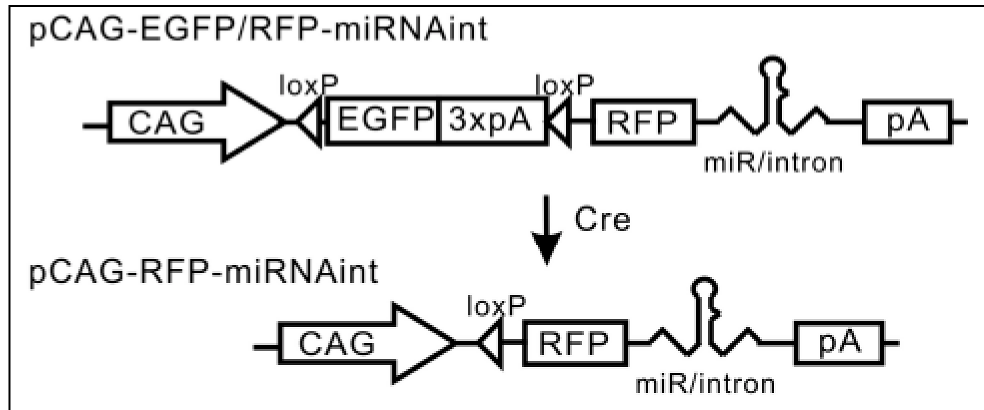


Figure 3: Irreversible conditional system involving the expression of the miRNA after the addition of Cre; provided by (Xu, 2008)

PROJECT PURPOSE

In order to analyze the effect of the loss of progranulin, an induced silencer needed to be transfected *in vitro*. NSC-34 neural cells were used to observe the function of progranulin in neurite growth, survival, and apoptosis. The first action needed was to induce knockdown. The hypothesis was that by producing a stable transfected cell line, 50% knockdown of mRNA for progranulin could be acquired. This was tested through RNA extraction and northern blots. Once stable cell lines with at least 50% knockdown were confirmed, the effect of progranulin could be observed.

In addition, the phenotype of a haploinsufficiency of progranulin was developed through the analysis of transgenic mice. Polymerase Chain Reaction was used to identify the mice expressing the miRNA that knocks down progranulin. The HomeCageScan System and the rotarod systems were used to examine the motor coordination and behavioral function of the transgenic mice. The hypothesis was that the mice with the knockdown of progranulin would not exhibit as high of motor function as the wild mice. For the HomeCageScan System, it was predicted that the transgenic mice would not travel as far as the wild mice and would spend a higher fraction of time resting as opposed to energetic behaviors. For the rotarod test, it was predicted that the transgenic mice would not be able to remain on the rod for as long as the wild mice. The loss of progranulin would decrease the motor coordination and function.

MATERIALS AND METHODS

Transgenic Animal Model

Polymerase Chain Reaction (PCR)

To verify the presence of the transfected DNA construct in the transgenic mice, two process were completed. First, the tail sample of each mouse was observed under green fluorescent light. If the transgene was incorporated into the mouse genome and was active, then the sample tail would appear to be fluorescent green due to the expression of the EGFP marker in the construct.

In order to confirm the existence of the miRNA, the polymerase chain reaction (PCR) was performed. This procedure amplified the DNA construct containing the EGFP and miRNA genes if it was present in the mouse sample. The first step was to digest the mice tail samples by rotating the tubes in lysis reagent overnight as according to the DirectPCR Lysis Reagent (Tail) procedure provided by Viagen Biotech, Inc (Viagen Biotech, Inc., 2007).

Once the tails were digested, the PCR reagent was mixed by combining the necessary components with the appropriate concentration according to Table 1. Twenty micro liters of the PCR reagent was added to each labeled tube of one micro liter of DNA sample. The samples were then put in the Peltier Thermal Cycle set to a program that consisted of 94˚C for 2 minutes, 33 cycles of 94˚C for 30 seconds, 64˚C for 30 seconds, and 72˚C for 30 seconds, followed by 72˚C for 5 minutes, and 4˚C until needed.

Table 1: Component and Concentrations for PCR reagent mix

Component	conentration PER sample (µl)
dH_2O	12.5
5X GoTaq Flexi Buffer	4
$MgCl_2$ solution, 25mM	2
dNTP (nucleotide), 10mM each	0.4
Forward Primer	0.4
Reverse Prime	0.4
GoTaq DNA Polymerase, 5µ/µl	0.3
Total	20

While the thermal system was finishing, an agarose gel was made. The samples were then placed in the electrophoresis gel consisting of 2% agarose in 1X TAE and ~5 µl of Ethidium Bromide. The electrophoresis was then run at 100V for ~20 minutes. Photos of the samples in the gel were taken using ultraviolet light. Dark bands represented the presence of the target DNA in the cells.

Rotarod

The rotarod is a treadmill-like machine that analyzes the motor coordination and fatigue of the mice. It consists of a rotating rod that gradually increases in rpm until the mice all fall off. The bottom of the machine is touch-sensitive, so once the mice hit the bottom, the timer stops. The recorded time that the mice were able to remain on the rotating rod is related to the motor functions.

HomeCageScan System

The HomeCageScan System is a video-based approach that recognizes behavior. Two mice at a time were placed in separate cages with fixed cameras for a period of around four hours. The cameras were connected to the HomeCageScan System program on the computer, where the movements and behavior of the two mice were categorized (Steele, Jackson, King, & Lindquist, 2007).

At the end of the session, a detailed analysis of the seconds and fraction of time spent for each behavior group was exported onto an excel sheet. The data for each set of mice was then further examined through graphs and statistical analysis. The behaviors of the double construct and wild type mice were compared.

***In vitro* Progranulin Knockdown**

Antibiotic Sensitivity

The cells that were cultured for the *in vitro* progranulin knockdown were NSC-34 cells, which is a motor neuron-like hybrid cell line. It is a hybrid line composed of motor neuron embryonic spinal cord cells and mouse neuroblastoma, which are cancer cells (CELLutions Biosystems, Inc., 2006).

The first step in developing the stable cell line was to culture the NSC-34 cells and determine the antibiotic sensitivity. It is necessary to know the minimum concentration of G418 that will kill the nontransfected cells, leaving the transfected cells containing the resistant gene

remaining. The procedure for determining the antibiotic sensitivity was followed according to the protocol of Transfection with pTK-neo (Novagen, 2003).

A necessary component to determine the antibiotic sensitivity was to observe the percentage of survival of the cells. The method to establish the number of viable cells in the cytotoxicity assay was followed according to the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay protocol (Promega, 2007).

Transfection

Once the antibiotic sensitivity was determined, the cells were transfected with a net solution containing the target DNA construct, Lipofectamine 2000, and pTK-neo plasmid according to Lipofectamine 2000 Plasmid DNA Transfection. The lipofectamine 2000 is lipid-based and allows the passage of the target DNA through the cell plasma membrane by binding to the DNA (Invitrogen, 2006). The pTK-neo plasmid allows the transfected cells to be resistant to G418 through the neomycin resistant gene (Novagen, 2003).

Stable Cell Line

When the cells were transfected with the target DNA, only some of the cells incorporated the DNA into their genome. For accurate results, it is necessary to develop a stable cell line, with 100% transfected NSC-34 cells. This was done in concurrence to the procedure of Transfection with pTK-neo (Novagen, 2003).

RNA Extraction

To verify the stable cell line, two methods were used. First, the cells were observed under a green fluorescent light. If the cells had incorporated the target DNA, then these cells would glow bright green. This is the result of the EGFP fragment of the target DNA construct.

Secondly, the miRNA, which knockdown the progranulin protein, is verified through the northern blot. An essential step before the northern blot is RNA extraction. Once cells have been cultured to a certain concentration, the RNA was isolated according to the RNA STAT-60 Procedure (Tel-Test). A small sample of the isolated RNA was used to determine the optical density, or the concentration of RNA per sample, so that equal loading could be used in the northern blot.

Northern Blot

With the RNA isolated from the sample cells, a northern blot was performed in order to detect the presence of mRNA for progranulin. Supposedly, the noninduced cells would be positive and the induced cells would be negative, because of the expressed knockdown of progranulin.

The gel was prepared by adding ambion agarose-LE to DEPC-treated distilled water. The NorthernMax 10X Denaturing Gel Buffer was added and allowed to solidify. In the meantime, the RNA samples were prepared by adding RNA samples, DEPC-treated distilled water, and formaldehyde load dye. It was denatured and then electrophoresed at 70V for 1.5 hours. The gel was photographed in order to confirm equal loading and then transferred to a BM nylon membrane.

The RNA was fixed on the membrane by UV crosslinking. This way, there was no loss of RNA. In order to view only the mRNA for progranulin, dig-labeled RNA probes were synthesized with SP6/T7 Transcription Kit (Roche). The membrane was then hybridized with the probe at 50˚C overnight, followed by two washes at 50˚C for 15 minutes. The northern blot images were developed with the LAS-3000 imaging system and quantified by the Multi-Guage software (FujiFilm).

RESULTS

In order to analyze the effect of the knockdown of progranulin in mice, the knockdown must first be verified. This was done through Polymerase Chain Reaction. Figure 4 shows the results of eight mice with the ladder on the left. The dark bands represent the positive transgenic mice that exhibit the miRNA and therefore express the RNA to knockdown progranulin mRNA.

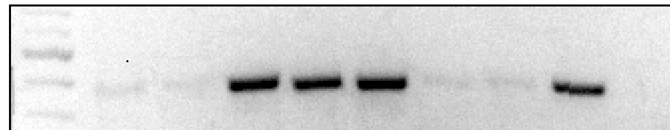


Figure 4: PCR of tail samples

After confirming the double construct mice from the wild type mice, behavioral tests were performed so that a phenotype could be established. The HomeCageScan System was used to analyze the behavior of the mice over a period of ~4 hours during the afternoon. For each session, a wild type mouse and a double mouse was put into the system. The averages for the wild type and double mice were calculated for the number of seconds and the fraction of time spent during each behavioral group. The results are displayed in Table 2.

Table 2: Results of the HomeCageScan System

		Average for Double Mice	Average for Wild Mice	Average for Double Mice	Average for Wild Mice
Group Behaviors	Member Behaviors	Seconds		Time Fraction	
Cage Top Activity	Hang Cuddled,Hang Vertically,Hang Vertically From HangCuddled,Hang Vertically From Rear Up,Remain Hang Vertically,Remain Hang Cuddled,Land Vertically	202.316	1005.192	1.24%	7.06%
Exploratory	Stretch Body,Forage,Dig,Sniff	504.764	1351.78	3.43%	9.22%
Feeding	Eat,Drink,Chew	373.332	1410.154	2.33%	9.51%
Locomotion	Walk Slowly,Walk Left,Walk Right	575.596	851.584	3.89%	5.72%
Rearing	Rear Up,Come Down,Come Down From Partially Reared,Come Down To Partially Reared,Rear up From Partially Reared,Rear up To Partially Reared,Remain RearUp,Remain Partially Reared	299.646	802.456	1.93%	5.47%
Resting	Stationary,Pause,Sleep,Remain Low,Twitch,No Data	10595.296	7775.79	68.91%	49.80%
Other		2720.11	2036.02	18.25%	13.21%

Another vital component to the HomeCageScan System is that it records the distances traveled for each mouse in meters. This is beneficial for the analysis of the loss of functional progranulin because it could represent fatigue and low engery. The distance traveled for each mouse and the averages for the double and wild mice are presented in Table 3.

Table 3: Comparison of the distance traveled in meters for wild type mice and double mice in the HomeCageScan System

	Gender	Distance Traveled (meters)
Cage 4B7I #7; Double	Male	34.98
Cage 5B7H #35; Double	Female	47.81
Cage 4B4H #1; Double	Male	35.9
Cage 4B5G #30; Double	Female	32.99
Cage 5B1K #22; Double	Female	118.11
Cage 4B7I # 8; Wild	Male	29.16
Cage 5B7H #32; Wild	Female	96.79
Cage 4B4H #2; Wild	Male	186.02
Cage 4B5G #2; Wild	Female	154.62
Cage 5B1K #27; Wild	Female	130.08

Average Distance Traveled (meters) for **Double** Mice	53.958
Average Distance Traveled (meters) for **Wild** Mice	119.3339

A column graph was developed in order to visualize the differences in the fraction of time spent performing each behavior. The wild type and double mice were compared and analyzed for correlations with the loss of the progranulin function. Figure 5 illustrates the comparison of the two mice groups. As shown, the double construct mice spent a greater fraction of time resting as opposed to the wild type mice.

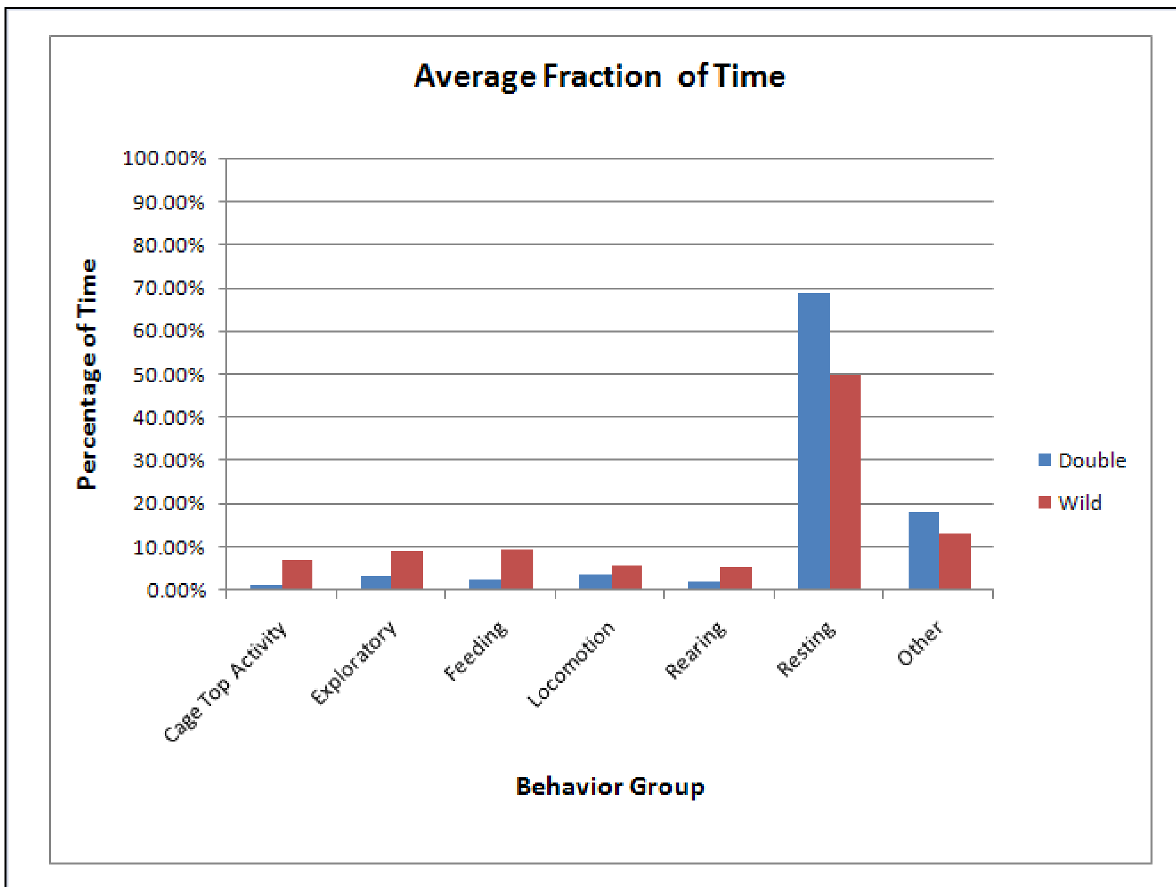


Figure 5: Graph of the percentage of time the double mice and wild type spent for each behavior group in the HomeCageScan System

The rotarod test was used to further analyze the motor function of the mice. The results of the wild type and double mice were compared for different age groups. The rotarod test was

performed at 2, 4, 6, 8, and 10 months of age for the mice and the averages of the results for each age group were calculated. Table 4 displays the average time for each group of mice.

Table 4: Results of the rotarod test (Average time in seconds)

	Number of Mice			
Age (months)	Double	Wild	Double	Wild
2	6	7	27.11	58.36
4	2	3	17.50	69.89
6	1	1	24.33	126.08
8	1	1	3.67	80.33
10	1	1	22.00	94.00

The data from the rotarod test was then analyzed further. A line graph was used to demonstrate the differences in the time remaining on the the rotarod for the double and wild mice in Figure 6. The results reveal that, on average, the wild type mice remained on the rotarod for longer amount of time than the double construct mice.

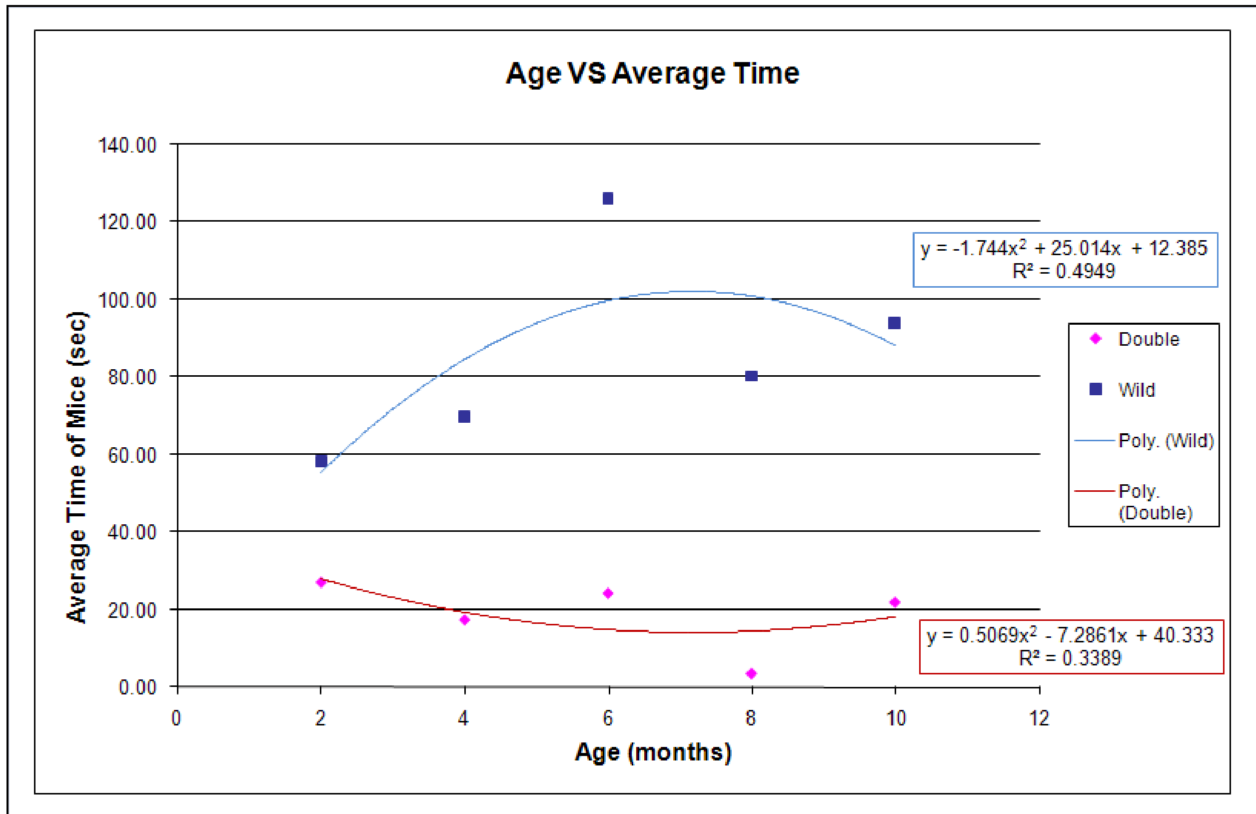
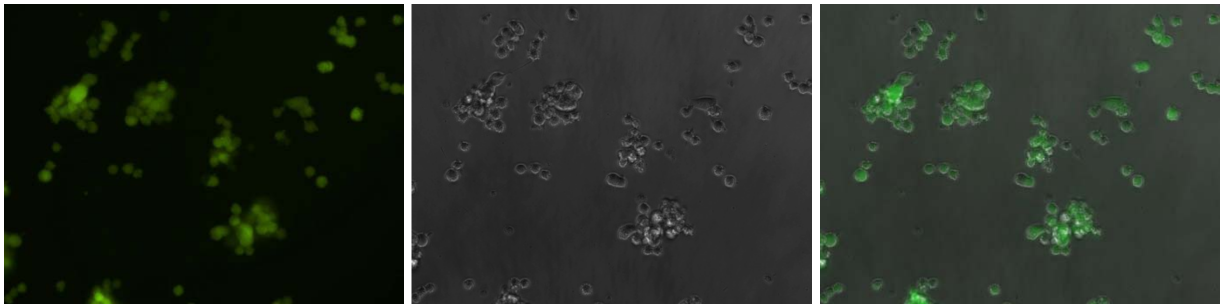


Figure 6: Graph of results from rotarod test illustrating the average time for the different ages

The first method that was used to confirm the incorporation of the target DNA construct into the cells was green fluorescent light. The cells were placed under a microscope at 20X magnification with green fluorescent light. If the cells contained the target DNA in their genome, then they would fluoresce green. Figure 7a displays a positively transfected stable cell line. Figure 7b shows the same cells under normal light, and Figure 7c is a photo combining the cells under green fluorescent light and the cells under normal light. This allows for any cells that are not expressing the EGFP to be visible. Figure 7c shows that all the cells are green, which means that this cell line was 100% stable.



(a) **(b)** **(c)**

Figure 7: Transfected cell line magnified at 20X showing (a) expression of Enhanced Green Fluorescent Protein (EGFP) under green fluorescent light, (b) same cells under normal light, and (c) combined photo in order confirm 100% transfection.

The second method that was performed was the northern blot to validate the presence of the miRNA. For accuracy, equal loading was necessary. Figure 8 displays the eight cell samples that were used. As seen in the photo, the darkness of the ribosomal RNA bands is equal, meaning equal loading was used in the northern blot.

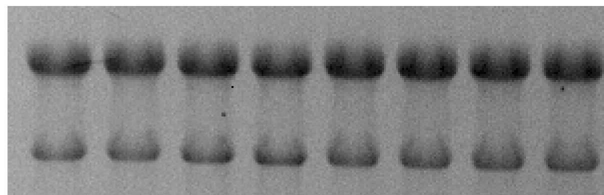


Figure 8: Equal loading for northern blot

The next step was to transfer the RNA downward to the BM nylon membrane and use premade dig-probes to target the mRNA for progranulin. The images were produced using the LAS-3000 imaging system. Figure 9 displays the results of the northern blot of the eight cell

samples. As shown, samples 1, 3, 5, and 7 represent brighter bands, which means that these cells have a higher quantity of mRNA for progranulin.

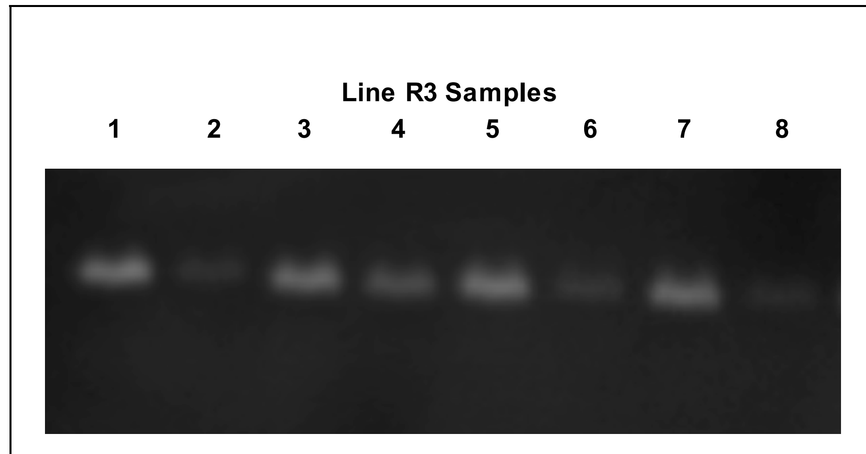


Figure 9: Results of nothern blot for R3 cell lines under 8 minutes of exposure

The samples are defined in Table 4. The samples with the brighter bands were in fact cells that were not induced, meaning they should have contained a higher number of mRNA for progranulin. The data was quantified using the Multi-Guage software (FujiFilm). The intensity of the bands was used to calculate the knockdown efficiency of the cell lines. The results are summarized in Table 5.

Table 5: Knockdown Percentage at mRNA Level for noninduced VS induced cells

Sample	R3 Line		Intensity (Area Under - Background)	Knockdown Efficiency (1-2)/1
1	R341-5	Control	15,433,469	82.31%
2	R341-5	Induced	2,730,464	
3	R356-5	Control	15,198,504	39.19%
4	R356-5	Induced	9,242,124	
5	R345-10	Control	17,686,258	73.27%
6	R345-10	Induced	4,726,653	
7	R345-4	Control	14,815,493	93.47%
8	R345-4	Induced	967,840	
B			0	
B			528,401	

DISCUSSION

This project consisted of two separate methods of analyzing the role of progranulin in FTD. Transgenic mice were used to develop an appropriate phenotype for the haploinsufficiency of progranulin. By comparing the behavior and motor functions of the transgenic (double) mice with the wild mice, the data could be used to examine the exact effect of the loss of progranulin and how its function in FTD.

The data generated from the HomeCageScan System supported the hypotheses that the transgenic mice would spend a higher fraction of time resting and the distance traveled in meters would be lower. On average, the transgenic double mice traveled 53.96 meters whereas the wild mice traveled 119.33 meters. The transgenic mice also spent the majority, 68.91%, of the time resting. The wild mice rested 49.8%, however, they were very active compared to the transgenic mice. The wild mice spent a greater fraction of time with cage top activity, exploratory, feeding, locomotion, and rearing.

From the rotarod test, it was concluded that the transgenic double mice were not able to remain on the rod as long as the wild mice. From ages two months to ten months, the average time in seconds that transgenic mice generated ranged from 3.67 seconds to 27.11 seconds. The wild mice ranged from 58.36 seconds to 126.08 seconds from ages two to ten months. As predicted, the loss of progranulin severely effected the motor function as observed by the rotarod test.

The second method used to examine the role of progranulin in FTD was inducing knockdown *in vitro*. Through RNA extraction and northern blots, the knockdown of the stable transfected cell lines was verified. A successful cell line was defined to have at least 50%

knockdown of mRNA for progranulin. The cell lines that did not reach this expectation were discarded, and only those cell lines with at least 50% knockdown would be used for further morphological analysis. The positive cell lines that met the knockdown expectation ranged from 73.37% to 93.47% knockdown of mRNA for progranulin.

The data that was generated through the analysis of the transgenic mice and *in vitro* knockdown is supported by other journal articles previously published. “The Neuropathology of Frontotemporal Lobar Degeneration Caused by Mutations in the Progranulin Gene” by Mackenzie, et al. concludes that an important cause of familial FTD is the null mutation of progranulin. Their studies explain how the there is linkage to chromosome 17q21 in families with FTD without identifiable MAPT mutations (Mackenzie, et al., 2006).

As shown by the data from the behavioral tests, the loss of progranulin severely affects the motor neuron function of the mice. This is supported by the journal article “Progranulin Functions as a Neurotrophic Factor to Regulate Neurite Outgrowth and Enhance Neuronal Survival” by Van Damme, et al., which examined the function of progranulin. The experiments performed by Van Damme, et al. revealed that progranulin is a neurotrophic factor that has a positive influence on neuronal survival and axonal outgrowth. The authors from the article concluded that the lack of progranulin through mutations leads to neurodegeneration (Van Damme, et al., 2008).

As suggested by Eriksen and Mackenzie in “Progranulin: Normal Function and Role in Neurodegeneration”, further research is needed to understand the exact mechanism by which the loss of functional progranulin leads to neurodegeneration as seen in patients with FTD. More

knowledge is needed of the trophic properties of progranulin in order to determine the role of progranulin mutations in these disorders (Eriksen & Mackenzie, 2008).

Future experiments involving the transgenic mice include a more detailed analysis of the behavior at varying ages. The results from the HomeCageScan System did not take into consideration the age or sex of the mice. As for the rotarod test, the mice used in this experiment will need to be further observed as they continue to age. The data from the rotarod test will be used to understand the long-term affect of progranulin on the motor neuron coordination of the mice.

Having verified a stable transfection with at least 50% knockdown of mRNA for progranulin *in vitro*, future research involves observing the morphological changes. The cells will be cultured in special medium, allowing them to differentiate. The induced cells will then be compared to the non-induced cells in order to understand the function of progranulin. The three factors to be examined as the cells differentiate would be survival, neurite growth, and apoptosis.

Depending on the results, further research could include knockdown in primary neuron cells as opposed to the NSC-34 cells. This experiment would represent the true effect of the loss of progranulin in cells in the human body.

The recent discovery of progranulin mutations as a cause of FTD has explained the linkage of chromosome 17q21 without the MAPT mutations. The results of this project involving the transgenic mice as well as the *in vitro* knockdown support this discovery. Although the exact mechanism of the haploinsufficiency of progranulin is still unknown, this project as well as previously published ones is narrowing down on the role of progranulin mutations in FTD. The phenotype and morphological changes are becoming understood, and with further research,

therapeutic interventions for familial FTD with progranulin haploinsufficiency will progress (Eriksen & Mackenzie, 2008).

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APPENDIX

Mice Record

Cage	Line	S	Birth	No	Record	Age		8/25/08
5B8G	Pgrn-9#23#94/Nes-cre#46,47#52,54F2	F	9/19/07	1	79	341	5B6H	
	Pgrn-9#28#42/GFAP-CrM#54#47F2	F	9/24/07		83	336		
5B1K	Pgrn-9#23#24/Nes-cre#40,41#62,63#58F2	F	12/5/07	3	22,24,27	264	5B7J	
4B5L	Pgrn-9#23#24#77#52/Nes-Cre#91#32#55,57,F4	F	3/21/08	3	11,12,13	157	5B3G	
4B5G	Pgrn-9#23#24#77#53/Nes-cre#94#60#35,39,F4	F	3/23/08	2	29,30, 2	155	5B5L	
4B6L	Pgrn-9#23#24#77#52/Nes-cre#55,57F4	F	4/13/08	1	L1	134	*5B3G*	
	Pgrn-9#23#24#77#53/Nes-cre#3,14F4	F	4/15/08	3	13,14,15	132	5B6I	
5B7K	Pgrn-9#23#24#77#53/Nes-cre#3,14F4	F	5/8/08	4	21,22,23,24	109	5B6I	
5B7H	Pgrn-9#23#24#8/Nes-Cre#21,22, F3	F	5/8/08	4	32,33,34,35	109	5B5G	on hold.
4B7K	Pgrn-9#23#24#6/Nes-cre#94#60#35,39,F3	F	5/21/08	4	9,10,11,12	96	5B5L	
5B2G	Pgrn-9#23#24#8#27/Nes-cre#92#30#36#3,F4	F	5/26/08	3	16,17,18	91	5B4I	
	Pgrn-9#23#94#58/Esr-Cre#4,38, F3	F	5/25/08	1	R2	92	5B3L	
5B9J	Pgrn-9#23#24#39/Nes-Cre#18,68, F3	F	6/3/08	3	8,9,10	83	5B5K	

Age Group	Days
2 months	45-75
4 months	105-135
6 months	165-195
8 months	225-255
10 months	285-315

Rotarod Test

Mouse #	Cage	Sex	Date of Birth	Age at Trial: Trail 1	Trail 2	Trail 3	Trail 4	Trail 5	Trail 6	Trail 7	Trail 8	Trail 9	Trial 10		
				5/28/08	6/3/08	6/10/08	6/12/08	6/17/08	6/19/08	6/26/08	7/1/08	7/23/08	8/5/08	8/15/08	9/17/08
22	5B1K	F	12/5/07	175	181	188		195			209		244	254	287
27	5B1K	F	12/5/07	175	181	188		195			209		244	254	287
30	4B5G	F	3/23/08	66	72	79		86			100		135	145	178
29	4B5G	F	3/23/08	66	72	79		86			100		135	145	178
2	4B5G	F	3/26/08	63	69	76		83			97		132	142	175
79	5B8G	F	9/19/07	252	258	265		272			286				
83	5B8G	F	9/24/07	247	253	260		267			281		316	326	359
11	4B5L	F	3/21/08	68	74	81		88			102		137	147	180
12	4B5L	F	3/21/08	68	74	81		88			102		137	147	180
1	4B6L	F	4/13/08				60		67	74		101	114	124	157
15	4B6L	F	4/15/08				58		65	72		99	112	122	155
13	4B6L	F	4/15/08				58		65	72		99	112	122	155
14	4B6L	F	4/15/08				58		65	72		99	112	122	155
24	5B7K	F	5/8/08				35		42	49		76	89	99	132
22	5B7K	F	5/8/08				35		42	49		76	89	99	132
32	5B7H	F	5/8/08				35		42	49		76	89	99	132
35	5B7H	F	5/8/08				35		42	49		76	89	99	132
12	4B7K	F	5/21/08										76	86	119
11	4B7K	F	5/21/08										76	86	119
10	4B7K	F	5/21/08										76	86	119
17	5B2G	F	5/26/08										71	81	114
18	5B2G	F	5/26/08										71	81	114
16	5B2G	F	5/26/08										71	81	114
2	5B2G	F	5/25/08										72	82	115
8	5B9J	F	6/3/08										63	73	106
10	5B9J	F	6/3/08										63	73	106
9	5B9J	F	6/3/08										63	73	106

Age Group	Days
2 months	45-75
4 months	105-135
6 months	165-195
8 months	225-255
10 months	285-315

TO BE TRAINED

	Mouse ID	Cage	Trials										Average	Total Average
2 Months	30	4B5G	23	18	30	5	16	15					17.83	
	12	4B5L	10	12	6	7	15	11					10.17	
	1	4B6L	3	15	6	21	3	3	5	4	12	21	9.30	
	15	4B6L	34	84	74	96	36	90	112	57	99	115	79.70	
	24	5B7K	27	8	9								14.67	
	35	5B7H	32	22	39								31.00	27.11
	12	4B7K												
	17	5B2G												
	18	5B2G												
	8	5B9J												
	10	5B9J												
	11	4B5L	111	118	108	86	100	55					96.33	
	13	4B6L	39	46	74	62	52	70	80	46	76	61	60.60	
	14	4B6L	54	47	40	33	53	70	86	71	34	48	53.60	
	10	4B7K												
	29	4B5G	22	26	24	61	44	67					40.67	
	9	5B9J												
	22	5B7K	69	67	44								60.00	58.36
	32	5B7H	19	48	40								35.67	
	11	4B7K												
	2	4B5G	59	54	39	95	52	71					61.67	
	16	5B2G												
	2	5B2G												
4 Months	30	4B5G	20	19	27								22.00	
	12	4B5L	20	9	10								13.00	
	1	4B6L												
	15	4B6L												
	24	5B7K												
	35	5B7H												17.50
	12	4B7K												
	17	5B2G												
	18	5B2G												
	8	5B9J												
	10	5B9J												
	10	4B7K												
	13	4B6L												
	14	4B6L												
	9	5B9J												
	29	4B5G	56	22	43								40.33	
	11	4B5L	96	106	117								106.33	69.89
	22	5B7K												
	32	5B7H												
	16	5B2G												
	2	5B2G												
	11	4B7K												
	2	4B5G	46	73	70								63.00	

6 Months	ID	Code													Average	Total
	22	5B1K	23	25	28	22	33	26	14	16	25	33	16	31	24.33	24.33
	12	4B5L														
	30	4B5G														
	29	4B5G														
	11	4B5L														
	2	4B5G														
	27	5B1K	83	114	80	168	154	144	138	150	119	135	88	140	126.08	126.08

8 Months	ID	Code													Average	Total
	79	5B8G	7	3	1										3.67	3.67
	22	5B1K														
	27	5B1K														
	83	5B8G	94	102	78	79	67	62							80.33	80.33

10 Months	ID	Code													Average	Total
	79	5B8G	7	24	35										22.00	22.00
	22	5B1K														
	27	5B1K														
	83	5B8G	108	68	106										94.00	94.00

HomeCageScan System

	Cage 4B7I #7; Double	Cage 5B7H #35; Double	Cage 4B4H #1; Double	Cage 4B5G #30; Double	Cage 5B1K #22; Double	Cage 4B7I # 8; Wild	Cage 5B7H #32; Wild	Cage 4B4H #2; Wild	Cage 4B5G #2; Wild	Cage 5B1K #27; Wild	Average for Double Mice	Average for Wild Mice
Group Behaviors	Time Fraction										Time Fraction	
Cage Top Activity	1.17%	0.02%	0.00%	5.02%	0.00%	0.25%	2.59%	23.04%	5.65%	3.75%	1.24%	7.06%
Exploratory	1.74%	2.36%	1.89%	3.25%	7.93%	1.06%	5.25%	10.83%	13.67%	15.31%	3.43%	9.22%
Feeding	2.82%	3.37%	0.89%	4.08%	0.51%	1.20%	7.07%	14.35%	15.22%	9.72%	2.33%	9.51%
Locomotion	2.33%	3.90%	2.23%	2.66%	8.35%	2.24%	5.67%	8.61%	6.23%	5.86%	3.89%	5.72%
Rearing	1.87%	2.38%	1.07%	2.62%	1.71%	0.78%	4.66%	7.80%	6.53%	7.58%	1.93%	5.47%
Resting	77.68%	73.47%	66.07%	66.20%	61.15%	77.14%	60.35%	26.03%	36.09%	49.40%	68.91%	49.80%
Other	12.39%	14.50%	27.85%	16.18%	20.35%	17.33%	14.41%	9.35%	16.60%	8.37%	18.25%	13.21%