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About The Exley



f Manchan Dean Blanchard Dean of Undergraduate Education

Dear Readers, As you know, many UT Dallas undergraduate students participate in research activities. Our students interact with the University's faculty, graduate students and postdoctoral professionals. Some undergraduates enroll in courses or participate in programs that provide an opportunity to demonstrate their creative talents. *The Exley*, UT Dallas' undergraduate research journal, supports this process by encouraging students to publish their work where it can be appreciated by a larger community.

The Exley provides undergraduate students from every discipline an opportunity to publish substantive work that illustrates their creative ability or research skills. The Office of Undergraduate Education manages the journal and publishes each issue in collaboration with the Office of Research, the Office of Communications, administrators from our schools and the University's faculty and students.

The work published in *The Exley* recognizes the dedication of both the authors and their faculty research mentors. I hope these contributions inspire other students to engage in research and share their creative work.

I would like to personally thank Elizabeth Exley Hodge. Ms. Hodge dedicated 19 years to UT Dallas as a valued employee and has continued her contributions by graciously supporting this forum for undergraduates. The Office of Undergraduate Education is very grateful for Ms. Hodge's generosity and commitment to the University's continued excellence in undergraduate education and research.

The spring of 2011 found Ms. Elizabeth Exley Hodge making a generous donation to support the publication of UT Dallas' first interdisciplinary undergraduate research journal. Hodge's maiden name of Exley represents the rich history of her family. Her brother, John, searched records in Manchester and Halifax, England, in 1971, which revealed that the surname Exley was believed first to be Ecclesley, dating from 1245, meaning "Church Fields." The area where her great-great-grandfather was born now exists as Exley Hall in Yorkshire, England. Several in the current family have visited there. The journal was named *The Exley* to show the University's appreciation of Hodge's support.

The Exley Name

Elizabeth Exley Hodge Biography

Hodge was born in a small farming community in Worcester County, Maryland, in 1920. She is one of eleven children of Lola Marie Watson and John O. Exley, who had distinguished himself by winning gold medals in rowing at the 1900 and 1904 Olympic Games. After high school, Hodge lived nine years in Philadelphia, where she worked for an insurance company. When World War II was declared, she volunteered in a program with the U.S. Air Corps, where she met the man she later married, Noble H. Hodge, who was from Fannin County, Texas. They were married in 1942. Following his military service in England, they moved in 1945 to Dallas, Texas, where Hodge still resides. In 1967, Hodge joined the administrative offices of the Southwest Center for Advanced Studies. When the center became UT Dallas in 1969, she transferred to the Department of Biology in the School of Natural Sciences and Mathematics, where she assisted faculty members preparing research grant applications. After a number of years in grants management in the School of Natural Sciences and Mathematics and later in the Office of Sponsored Projects, she retired in 1986. Hodge has been an avid gardener for many years. She has a personal arboretum and an orchid hybrid that bears her name. She enjoys cooking and sharing her time with others. Hodge has volunteered weekly for the last 23 years at Baylor Medical Center in Garland. She is a member of St. John's Episcopal Church near her home.





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About the research contributors



Ardalan "Ardy" Sharifi was born in Iran, came to the United States at the age of 15, and is now a junior biology major with a minor in neuroscience at UT Dallas. While serving as a summer research intern for the Louis Stokes Alliance for Minority Participation, he became intrigued by the world of enterococci and their cellular defense system, CRISPR-Cas. Outside of school, he enjoys cooking, bird watching and playing video games. Currently, Ardy researches in Dr. Kelli Palmer's lab and volunteers weekly at Allen Presbyterian Hospital. Upon graduating, he hopes to attend medical school and pursue his dream of becoming a cardiologist.

tel is a senior pursuing a degree in neuroscience. He began his elementary schooling in Chennai, India, and Shrinath later immigrated to the United States to complete his secondary education in Houston. He developed an early interest in the human brain, intrigued by its relationship with behavior and psychology. While in high school, Shrinath was involved in neuroscience research, studied aging at Baylor College of Medicine, and volunteered in an Alzheimer's patient facility to better understand current treatment options for mental health and brain-related illnesses. While at UT Dallas, Shrinath joined Dr. Sven Kroener's lab, where he studied alcohol addiction and schizophrenia in animal models. He currently researches human cognition and behavior at the Center for Brain Health, under Dr. Francesca Filbey. Shrinath enjoys giving back to the community by volunteering, promoting sustainable living, and fostering health and well-being. To give back to the world and make a direct difference in people's lives, Shrinath hopes to work as a physician. He extends his deepest gratitude to his family, friends and academic mentors, for all their help, both in school and in life.

ao Duong is a junior marketing and business administration major from Hanoi, Vietnam, and has long been aware of health care, income inequality and education problems. Thao is also interested in cultural exchange, and in the fall of 2013, she participated in an intensive language program in China, completing her minor in Asian studies. She is currently a member of AIESEC, a global youth-led organization that promotes international exchanges for students and recent graduates. She hopes to one day utilize her educational background, research on social welfare in the United States, and study abroad experiences to tackle current challenges in Vietnam.



Ngo is a senior management student from Hanoi, Vietnam, majoring in economics and finance with a minor in performing arts. Her research interests were inspired by classes taught by passionate School of Economic, Political and Policy Sciences faculty and the friends she met at the 2011 New Economy Conference. Amy has been working in the Fair Park Neighborhood Study under the supervision of Dr. Tammy Leonard since the fall of 2012. Through volunteering with local food banks and the Salvation Army, she developed an interest in improving living conditions for the needy, compelling her published research on identifying causes of an unhealthful diet. Amy has been a member of Phi Kappa Phi Honors Society, Davidson Management Honors Program, Financial Leadership Association, the FSA modern dance team, and the UT Dallas dance ensemble. She aspires to one day work on Wall Street, and in her free time she enjoys running, trying out new recipes, and entertaining her friends with dinner parties.

Xiao Tan is a senior biochemistry student from Harbin, China, who arrived in the United States at age 10. She is currently working under Dr. Jin Wang at the Baylor College of Medicine, focusing on the development of a drug delivery method by applying Michael additions. She has worked under Dr. Gail Breen in exploring the role of reactive oxygen species in neurodegenerative tauopathies. Her research experiences also include the summer research program at the Baylor College of Medicine, and her hobbies include drawing, reading and writing. Upon graduation, she would like to continue her study of pharmacology, which she believes to be the perfect blend of chemistry and biology.

n is a junior physics major from Hanoi, Vietnam. He discovered his passion for physics after taking his first physics course at UT Dallas with Dr. Joseph Izen. The question of fundamental constituents of the universe and the prediction of solutions based on elegant ideas, such as symmetry and unification, render physics appealing to him. His commitment toward pursuing a career as a physicist was established during his summer research internship with EURECA, a dark matter search project, at Karlsruhe Institute of Technology in Germany. Currently, he is doing research in elementary particle physics with Dr. Izen at the SLAC National Accelerator Laboratory. Besides coursework and research, he works as a physics tutor at the Student Success Center and as a math tutor in the Math Lab. He enjoys ballroom dancing and is a jazz/classical music lover.



Collecting "Wild" Enterococci

Enterococci are commensal microorganisms that commonly colonize the intestines of humans, birds and other animals. These bacteria are opportunistic pathogens and can cause life-threatening infections in humans, especially in hospital environments where high levels of antibiotic resistance in certain strains contribute to their pathogenicity. Two species of this genus that opportunistically cause infections in humans are *Enterococcus faecium and Enterococcus faecalis*.

Most enterococcal strains used for laboratory studies are clinical isolates from human infections. The purpose of this study was to isolate novel enterococci from environmental (nonclinical) samples. This study is significant because it seeks to identify genetic or phenotypic characteristics that could be used to discriminate between clinical and environmental enterococci. The "wild" isolates collected from environmental samples will be compared to human infection isolates in future studies.

— by Ardalan Sharifi



Background

Enterococci are part of the normal intestinal flora of humans and animals, although some species can also opportunistically cause serious infections in compromised individuals.¹ The genus *Enterococcus* includes more than 17 species, but only a handful can cause clinical infections in humans.² With an increasing number of antibiotic-resistant enterococcal strains and a decreasing supply of new antibiotic classes, enterococci are specially recognized as opportunistic pathogens of concern.³



Figure 1. E. faecalis grown on BEA agar.

Enterococci are hardy, facultative anaerobic bacteria that can survive and grow in many different environments.⁴ In the laboratory setting, enterococci can be distinguished from other microorganisms by using BEA (Bile-Esculin-Azide) agar, a selective growth medium. Enterococci hydrolyze the glycoside, esculin, to esculetin. Esculetin reacts with an iron salt, ferric ammonium citrate, to form a dark brown or black complex.⁵ Azide in the medium prevents the growth of gram negative bacteria. Different enterococcal species can be distinguished from each other by screening for the presence of species-specific genes using the polymerase chain reaction (PCR). A previously described screening strategy uses primers targeting the *E. faecalis* and *E. faecium ddl* genes to identify and discriminate between the two species.⁶ Another rapid and low-cost method of bacterial identification is via 16S rRNA gene sequencing. Ribosomes are the universal machinery that translates the genetic code, RNA, into proteins. They have two subunits that associate upon the start of protein synthesis. All bacterial cells have a 16S rRNA gene that encodes ribosomal RNA. Ribosomal RNA is a significant and critical component of the ribosome. The 16S rRNA gene is a universal gene that is highly conserved among bacteria and is about 1,550 base pairs in length. The 16S rRNA molecule folds into exotic shapes in order to fit with other molecules of the ribosome.⁷ Since proper structure is the elemental requirement of any working biological component, the overall shape of 16S rRNA has stayed relatively the same over evolutionary time. Some sections of the sequence may vary, but overall, the bacterial 16S rRNA gene is highly conserved. The varying regions of 16S rRNA, which do not contribute much to its overall structure, are called the variable regions. The number of sequence variations in the 16S rRNA genes of different species can be correlated with the evolutionary distance between the species.8



Figure 2. Structure of 16S rRNA after posttranscriptional modifications.⁹

In this study, I collected environmental samples and incubated them on BEA agar to select for enterococci. Bacterial colonies arising on BEA agar were then screened using a PCR-based approach. The 16S rRNA genes for two enterococcal isolates were sequenced. Using this approach, I isolated two novel *Enterococcus hirae* strains.

Materials and Methods

Environmental sampling and selection for enterococci:

Water, flowers, rocks, bird fecal matter, grass and dirt were sampled from different sites on The University of Texas at Dallas campus (Richardson, Texas). A study carried out by Auckland University in New Zealand showed that enterococci can be found on degrading drift seaweed at recreational beaches with a much higher magnitude than their levels in seawater, suggesting they have the potential of contaminating adjacent water and sand.¹⁰ Therefore, water and degrading driftwood samples were also obtained from Hillcrest Estate Lake in Frisco, Texas. The samples were plated on BEA agar and incubated at 37°C. Plates were examined for bacterial growth after overnight incubation. BEA agar was purchased from Becton, Dickinson and Co., Sparks, Maryland.

Primer's Name	Primer Sequence	Preferred Annealing Temperature	Expected Size
1492R (16S rRNA)	5'+ GGTTACCTTGTTACGACTT	50—52°C	~1,500
8F (16S rRNA)	5' - AGAGTTTGATCCTGGTCAG	50—52°℃	base pairs
	5′ + GCAAGGCTTCTTAGAGA	50—52°C	
	5'- CATCGTGTAAGCTAACTTC	50—52°C	~330 bp
	5' + ATCAAGTACAGTTAGTCTT	50—52°℃	
	5'- ACGATTCAAAGCTAACTG	50—52°C	~941 bp

Table 1. List of primers used in this experiment.

Multiplex colony PCR:

Select colonies arising on BEA agar were screened by multiplex colony PCR. For multiplex PCR, two different primer sets were added to the same PCR reaction. One primer set consisted of universal 16S rRNA gene primers that should amplify a product from any bacterial strain. The 16S rRNA gene primer pair (8F and 1492R) is capable of amplifying nearly full-length 16S rRNA gene sequences. The second primer set targeted either the *E. faecalis or E. faecium ddl* gene. The primers used in this research are shown in Table 1. Primers were purchased from Sigma-Aldrich (St. Louis).

For the PCR reaction, Tag polymerase enzyme, forward and reverse primers, deoxyribonucleotides (dNTPs), and water were included in a final reaction volume of 25 µl. A boiled bacterial colony provided a DNA template for the PCR reaction. The PCR mixture was initially heated to 95°C for five minutes to denature the DNA into single strands. The temperature was then held constant at 95°C for 30 more seconds. The temperature was then lowered to 50°C to allow annealing of the primers. Next, the temperature was increased to 72°C, allowing the Tag polymerase to extend the new DNA strand. The last three steps were repeated 30 times. Finally, the mixture was held at 72°C for 10 minutes for a final extension, and then the temperature was lowered to 4°C to preserve copies of the DNA. DNA from laboratory strains of E. faecalis and E. faecium were used as templates for positive control PCR reactions. Reactions lacking a DNA template were used as negative controls. PCR products were visualized by agarose gel electrophoresis with ethidium bromide.



Figure 3. Multiplex PCR performed with 16S rRNA primers and E. faecalis ddl primers. This PCR shows that my samples do not belong to the E. faecalis family.

16S rRNA gene sequencing:

To classify my environmental isolates, I compared the 16S rRNA gene sequences of two isolates with the 16S rRNA gene sequences of four common species of enterococci. The 16S rRNA gene sequences of two enterococcal isolates were amplified using colony PCR as described above. The sample DNA was purified using the Qiagen MinElute PCR Purification Kit and was then sent to Massachusetts General Hospital for sequencing using the fluorescently labeled dideoxy-nucleotide chain termination method. The DNA sequence was analyzed using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov) of the National Center for Biotechnology Information (NCBI).



Figure 4. This figure in a quick summary shows the step-bystep method in recovering, identifying and characterizing "wild" enterococci from the environment.

Results

Overall, I recovered and screened 20 different isolates obtained from BEA agar. An overview of the method used for isolation and characterization of these isolates is shown in Figure 4. Data analysis showed that none of the isolates were *E. faecalis* or *E. faecium*. A representative result from multiplex PCR screening is shown in Figure 3. In this case, isolates obtained from flowers, bird fecal matter and soil were screened using 16S rRNA gene primers and *E. faecalis ddl* primers. The results show that each of the isolates were positive for 16S rRNA gene products but were negative for the *E. faecalis ddl* gene. From this, I could conclude that the isolates were not of the *E. faecalis* species.

The 16S rRNA gene sequences from two of my isolates were obtained by DNA sequencing. The 16S rRNA gene sequence has highly conserved and variable regions; for example, there are nine variable regions for *Escherichia coli* 16S rRNA.¹¹ These regions of the gene help in discriminating the genus and/or species, resulting in a useful and economical method to classify novel bacterial isolates.

Figure 5 shows that the 16S rRNA gene sequences of my two novel isolates are very similar to those of E. hirae and E. faecium. E. hirae is closely related to E. faecium, and in the scientific literature prior to the 1980s, it was actually called Streptococcus faecium.¹² It is phylogenetically related to E. faecium with 16S rRNA gene sequence identities of over 97.8 percent. With such high percentages of similarity, conventional sequencing techniques are not enough to fully differentiate E. hirae from E. faecium and can actually cause error in species identification.13 I was able to further confirm that my novel strains were not *E. faecium* by using ddl primers specific to E. faecium. In multiplex PCR reactions, my isolates were positive for 16S rRNA gene products but negative for *E. faecium ddl* gene products. From these results, I concluded that my enterococcal isolates of interest belong to the E. hirae species.



Percentage Similarity of my

Figure 5. Percentage similarity score between my two novel strains and other known enterococci. The similarity score was developed after looking at the nine variable regions of the 16S rRNA gene. This figure shows that my two samples are very similar to E. hirae and E. faecium. I further confirmed my strain is not E. faecium by using ddl primers specific to E. faecium 733. The percentage similarity score shows that my strains are in fact similar to E. hirae.

Discussion and Conclusions

In this study, I isolated novel enterococci from environmental samples collected in the Dallas area. Of the 20 isolates obtained, two were classified to the species level and were found to be *E. hirae* isolates. *E. hirae* are nonmotile, ovoid cells that occur mostly in pairs or short chains. Researchers have found that *E. hirae* is involved in growth depression of young chickens.¹⁴ In a study carried out by Kyoto Pharmaceutical University, researchers found out that heat-killed cells of *E. hirae* ATCC 9790 were able to protect

epithelial cells from impairment, thus maintaining epithelial homeostasis. These results suggest that the lipid-related active component present in the ATCC 9790 cell wall, lipoteichoic acid, is crucial for this effect. This study demonstrates that *E. hirae* is a potential probiotic bacterium in the human intestine.¹⁵

The long-term goal of this project is to compare "wild" enterococci isolates with human infection isolates and potentially find novel methods to discriminate between environmental and clinical enterococci. It is important to know more about enterococci in the environment because little research has been done in this area. Future investigation could further indicate the presence and characteristics of pathogenic strains that occur in environmental settings. In future studies, it will be of interest to identify more of my enterococcal isolates to the species level, as well as to determine the antibiotic susceptibilities and resistances of these isolates.

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Memory Among Adult Chronic Marijuana Users: Exploring Comorbid Effects of Smoking Nicotine

Marijuana, also known as cannabis, has historically been a substance of choice for its medicinal, psychoactive and recreational use. Today, marijuana maintains its prominence as the most widely used illicit drug in the world.¹ Discordance in enforcement policies for marijuana use is prevalent within the U.S.; marijuana is legalized for recreational use in two states, legalized for medicinal use in 20 states, and illegal throughout the rest of the country. These differing laws make it evident that the perceived effects of marijuana use remain incongruent.

Aside from the physiological effects observed in certain cases (e.g., impaired respiratory function, cardiovascular disease, etc.), the primary consequences of chronic marijuana use lie in neurocognition, particularly observed in the domain of memory. In a pioneering review of long-term marijuana use and memory effects, Solowij and Battisti emphasized that memory impairments remain a quintessential consequence of chronic marijuana use.² However, given the high comorbidity of marijuana and nicotine use (e.g., approximately 75 percent of marijuana users also use nicotine), limited literature exists concerning the effects of marijuana and nicotine on memory.³ Ramo and colleagues found that memory impairment in adolescents was exacerbated with co-use of nicotine and marijuana.⁴ This significant finding underscores the importance of determining if effects are compounded in adult marijuana users who use nicotine.

In this study, we aimed to characterize the specificity of marijuana's effects on memory function by comparing memory function in marijuana-only users, nicotine-only users, and comorbid marijuana and nicotine users. Additionally, we tested how these effects may be related to frequency of marijuana use.

— by Shrinath Kadamangudi

Methods

Participants:

We analyzed data from 109 heavy marijuana users, 36 control subjects, and 55 heavy nicotine users who took part in two separate larger studies. All participants were required to be between 18 and 50 years of age, to not have engaged in illicit drug use (besides marijuana) during the 60 days prior to testing, and to not have a history of traumatic brain injury, neurological disease, or psychosis. Marijuana users were included in the study if they reported current marijuana use (verified by urinalysis) of at least four times a week for the six months prior to testing. Nicotine users were included if they reported current nicotine use (verified via breath CO monitor) of 10 or more times daily and had fewer than three months of abstinence in the past year. Non-using controls were recruited with a corresponding age and strict no-drug-use criteria within the past year. The Substance Use Disorder (SUD) module of the SCID-II (Structured Clinical Interview for DSM-IV Axis II disorders) was administered by a trained research assistant to assess SUDs for alcohol, marijuana, cocaine, opiates, hallucinogens, stimulants, sedatives and other drugs.⁵ Marijuana and nicotine smoking patterns among individuals were acquired through the Cannabis History Questionnaire and Smoking History Questionnaire.⁶

Marijuana and nicotine users were administered tests of memory after required abstinence (e.g., three days for marijuana users and 12 hours for nicotine users) to control for any acute drug effects. We assessed differences in memory function between (1) marijuana users and non-using controls, (2) nicotine-only users and (3) marijuana + nicotine users (Table 1).

Outcome measures:

The Wechsler Memory Scale (Version III) is a standardized neuropsychological test of memory commonly utilized in clinical psychology and neuroscience research.⁷ We utilized the Logical Memory I and Logical Memory II sections of the WMS-III, which tests immediate and delayed verbal recall, respectively. In the Logical Memory I task, a trained research assistant read two different stories aloud, after which subjects were asked to recall the story immediately. In Logical Memory II, subjects were asked to recall both stories sequentially, to the best of their memory, after about 30 minutes of cognitive rest (no other neuropsychological battery was administered within this wait time). To score subjects on recognition, the research assistant read segments out loud; some segments were from story A, some segments were from story B and some segments were never mentioned in either story. Using a yes or no response format, subjects were asked to recognize if the segments were truly a part of the stories. Scores of the delayed recall and recognition tasks were combined to tabulate raw delayed memory performance. Raw immediate and delayed memory scores were crosstabulated on a standardized scoring system to yield scaled scores, which were utilized for analysis. Lastly, frequency of marijuana use information was acquired from the Cannabis History Questionnaire.

	Marijuana Only (n=70)	Non-Using Controls (n=34)	Marijuana + Nicotine (n=28)	Nicotine Only (n=31)
Age (Average years ± SD)	24.01 <u>+</u> 7.55	31.38 <u>+</u> 10.53	31.23 <u>+</u> 10.43	26.96 <u>+</u> 8.96
Male (n,%)	46, 65.71%	8, 23.53%	19, 67.86%	16, 51.61%
Female (n,%)	24, 34.29%	26, 76.47%	9, 32.14%	15, 48.39%
Total Years of Education (Average years ± SD)	13.46 ± 2.58	15.43 ± 2.39	13.76 ± 2.15	12.54 ± 1.94
IQ (Average <u>+</u> SD)	105.01 ± 17.94	114.42 ± 10.05	110.36 ± 11.59	105.00 ± 12.20
Frequency of MJ use [sessions per day] (Average <u>+</u> SD)	6.224 ± 1.28	NA	NA	NA
Frequency of Nic use [cigarettes per day] (Average <u>+</u> SD)	0.8231±2.13	NA	15.19 ± 7.94	14.76 ± 8.40

 Table 1. Information on average age, total years of education
 and IQ along with percentage of male and female subjects.

Statistical analyses:

First, we conducted tests of group equivalence for potential confounding variables, which may affect performance on memory task between groups besides smoking marijuana or nicotine, such as age, gender, total years of education and IQ (measured via Wechsler Adult Intelligence Scale). To that end, we performed a one-way analysis of variance (ANOVA) for each continuous variable of interest (age, total years of education and IQ). For discrete variables such as gender, a chi-square analysis was performed. Second, to determine differences in memory functioning across groups, we performed a 4 (marijuana only, non-users, nicotine only and marijuana + nicotine) x 2 (immediate and delayed memory) analysis of covariance (ANCOVA), controlling for variables that differed between groups (based on an initial equivalence test). Third, to determine directionality of memory effects due to smoking marijuana alone, nicotine alone or both combined, a post-hoc Tukey

test was performed. Finally, a correlation analysis was performed to test how frequency of marijuana use affects immediate and delayed memory.

Results

We tested for group equivalence for potential confounding factors; age, total years of education and IQ were tested via a one-way analysis of variance, and a chi-square test was utilized for gender. From the one-way ANOVA, total years education (p < 0.003) and IQ (p < 0.013) met significance; the chi-square test for gender also met significance (p < 0.001). This implies that these three factors significantly vary between groups. Thus, they must be controlled for when testing memory function due to smoking.



Immediate verbal memory recall:

Figure 1. Non-using control subjects scored significantly higher than all three smoking groups (μ control = 11.485 + 2.425). Nicotine-only smokers scored slightly higher than marijuana-only smokers (μ nicotine only = 10.679 + 3.186 < μ marijuana only = 9.184 + 2.713). Average immediate memory scores for combined marijuana and nicotine smokers were significantly lower than the other smoking groups (μ Marijuana + Nicotine = 8.04 + 3.19). A post-hoc Tukey test yielded significant differences between controls and marijuana-only smokers* (p < 0.001), controls and marijuana + nicotine smokers* (p < 0.001), and nicotine-only and marijuana + nicotine smokers* (p < 0.031).

We performed an ANCOVA to test the effect of smoking marijuana, nicotine or both on immediate memory scores. After controlling for variables that significantly varied between smoking groups (gender, total years of education and IQ), variance in immediate memory scores due to smoking proved to be significant (F = 3.145, p < 0.006).

Further, mean differences between controls and marijuanaonly smokers (μ control - μ marijuana only = 2.301; p < 0.001), controls and marijuana + nicotine smokers (μ control - μ marijuana + nicotine = 3.445; p < 0.001), and nicotine-only and marijuana + nicotine smokers (μ nictotine only - μ marijuana + nicotine = 2.639; p < 0.031) proved to be significant after performing a Tukey test.

Delayed memory recall:



Figure 2. Non-using control subjects scored significantly higher than all three smoking groups (μ control = 12.060 + 2.397) in the delayed memory task. Nicotine-only smokers scored slightly higher than marijuana-only smokers (μ nicotine only = 11.071 + 3.15 < μ marijuana only = 10.000 + 2.905). Average delayed memory scores remained significantly lower for combined marijuana and nicotine smokers (μ Marijuana + Nicotine = 8.680 + 3.198). A post-hoc Tukey test yielded significant differences between controls and marijuana-only smokers* (p < 0.005), controls and marijuana + nicotine smokers* (p < 0.002), and nicotine-only and marijuana + nicotine smokers* (p < 0.05).

We performed an ANCOVA to test the effect of smoking marijuana, nicotine or both on delayed memory scores. After controlling for variables that significantly varied between smoking groups (gender, total years of education and IQ), variance in delayed memory scores due to smoking proved to be significant (F = 3.463, p < 0.018). Further, mean differences between controls and marijuana-only smokers (µcontrol - µmarijuana only = 2.060; p < 0.003), controls and marijuana + nicotine smokers (µcontrol - µmarijuana + nicotine smokers (µcontrol - µmarijuana + nicotine smokers (µnictotine only and marijuana + nicotine smokers (µnictotine only - µmarijuana + nicotine = 2.391; p < 0.048) proved to be significant after performing a Tukey test.

Relationship with frequency of marijuana use:



Figure 3. Interaction between delayed memory scores and frequency of marijuana use (darker points indicate multiple memory scores for the same frequency of use). We observed a significant and relatively strong negative correlation (R = -0.220, p < 0.032) between frequency of use and immediate memory performance.

In our final analysis, we performed a linear regression analysis to examine if memory scores were modulated by frequency of marijuana use. We discovered a significant negative correlation (R = -0.220, p < 0.032) between frequency of use and immediate memory performance. However, we did not observe any significance or correlation (R = -0.120, p < 0.1159) between frequency of marijuana use and delayed memory performance.

Discussion and Conclusion

In our study of memory function among heavy marijuana users, we found profound deficits in immediate and delayed memory in comparison to non-drug-using control subjects. Additionally, we observed significant memory impairment in frequent co-users of marijuana and nicotine in comparison to non-using control subjects; however, this effect was nominally, but not significantly, exacerbated by the combined use of marijuana and nicotine in comparison to marijuana use alone. These effects were observed after considering polysubstance use (excluding currently abusive or dependent polysubstance users) and controlling for gender, total years of education and inherent intelligence differences, as well as acute effects (following abstinence). We also found that frequency of use showed a negative correlation with immediate, but not delayed, memory performance.

Effects of chronic marijuana use have been observed to cause neural inefficiency as well as use of poorer compensatory mechanisms in memory systems. In an electrophysiology study performed by Battisti and colleagues, a shift to greater positivity due to earlier event-related potential peaking (suggesting shorter latency of activation) in brain regions involved in memory (hippocampus and parahippocampus) was interpreted as a general failure to effectively activate these memoryrelated structures.⁸ To support this, neurophysiological and pharmacological studies have shown decreased activation in high cannabinoid density areas (e.g., hippocampus and nucleus accumbens) due to cannabinoid-induced reduction of neurotransmitter release in GABAergic interneurons.9 In imaging studies, reduced hippocampal volumes in chronic users have also been observed.¹⁰ Compensatory mechanisms, or a greater recruitment of surrounding structures, have been frequently observed in chronic marijuana users who display proper memory functioning.¹¹ The nominally exacerbated performance in the immediate and delayed memory tasks by the marijuana + nicotine smoking subjects in comparison to marijuana-only users could be attributed to an interference of these compensatory mechanisms due to combined use of marijuana and nicotine. For example, prior research has demonstrated the importance of working memory circuits for successful encoding during verbal memory formation.¹² During short periods of nicotine withdrawal (24 hours), yet sustained marijuana use, abnormal function of fronto-parietal cortical networks, a possible area of compensation, seems to compromise the efficacy of verbal encoding during verbal memory formation.¹³ Additionally, frequency of marijuana use values for co-users of marijuana and nicotine were unavailable; the slightly poorer performance displayed by the marijuana + nicotine smoking subjects could be attributed to an increased frequency of marijuana use in comparison to subjects who smoked only marijuana. Finally, the effects of cannabinoid residues and metabolite buildup (fat, liver, lung and spleen) in highly frequent marijuana users could potentially explain our finding of diminished immediate memory function as a result of more frequent marijuana use. Immediate memory was correlated with frequency of use; however, delayed memory was not. This could be explained by the idea that immediate memory may structurally and functionally resemble short-term working memory processes; short-term working memory has been repeatedly observed to be correlated with frequency of marijuana use.¹⁴ Given enough time to consolidate information, recall capability might be preserved. The neural mechanisms behind memory, which may be affected by smoking marijuana and nicotine, remain largely unstudied; this study paves the way for future research to focus on discovering these respective mechanisms.

Rising use of marijuana and nicotine, individually and combined, and across all age groups, necessitates better understanding of their specific and interactive effects.¹⁵ In the present study, we have observed significant impairment in memory, an essential facet of cognition, due to chronic marijuana use and its combined exercise with nicotine. Given our findings, it is important for drug enforcement policymakers as well as the general public to be aware of the cognitive effects of smoking marijuana and nicotine before readily accepting them for recreational or acclaimed therapeutic use.

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Understanding and Improving the Eating Habits of low-Income Households

Despite its status as one of the world's greatest economic superpowers, the United States is also home to millions of poor. According to the most recent U.S. Census Bureau figures, 15.9 percent of Americans lived below the poverty level in 2011.¹ Over the past several decades, the government has implemented different programs, many of which deal with hunger, to assist low-income households. For example, for the past 40 years, the Supplemental Nutrition Assistance Program (SNAP) offered by the U.S. Department of Agriculture (USDA) has dispensed monthly benefits to qualified households for the purchase of food items or seeds and plants to produce food. In fiscal year 2011, SNAP received \$78 billion in federal funding and reached its highest enrollment level.²

Big-box retailers, supermarkets and other grocery store formats are beginning to open stores in socioeconomically depressed neighborhoods to provide easier access to healthful and affordable groceries. Government assistance also funds certain school lunch and breakfast programs. In these programs, children of low-income households are eligible for free or reduced-price nutritious meals at public and nonprofit private schools as well as residential child care establishments. In 2011, school meal programs received \$14.1 billion in federal assistance.³

- by Thao Duong

Introduction

However, hunger and obesity represent the flip side of food insecurity, which is defined as the inability to obtain an adequate quantity and quality of food. In 2009–2010, more than one-third of adults and almost 17 percent of youth in the United States were obese.⁴ Obesity threatens public health with increases in the risk of heart disease, diabetes, high blood pressure, cancer and other chronic conditions, leading to significant health care expenses. On average, an obese individual pays \$1,429 more in annual health care costs than an individual of normal weight. Medicare, Medicaid and private insurers, respectively, pay \$1,723, \$1,021 and \$1,140 more annually for obese patients than for normal-weight beneficiaries.⁵ The occurrence of obesity is, for the most part, evenly distributed across socioeconomic groups: 32 percent of low-income and 21.7 percent of high-income Americans are obese.⁶ Thus, food insecurity and obesity are not mutually exclusive; in fact, the poor will often choose low-cost-per-calorie, unhealthful fast food as an easy way to meet daily calorie requirements. Low-income communities in the United States are in need of new economically effective approaches to resolve the obesity-hunger paradox.⁷

Our research investigates a crucial factor associated with the obesity-hunger paradox, which describes the propensity of low-income households to resort to eating sugar- and fat-laden fast food on a daily basis in order to get the biggest bang for their buck in terms of calories. Despite retailers' efforts to transform "food deserts" into "food oases" and SNAP notwithstanding, economically disadvantaged households still engage in unhealthful eating habits. School meal programs, though undeniably improving the quality of meals children receive during school hours, cannot deter households from making poor food choices outside of school.

By seeking to understand the unhealthful eating habits of low-income households, latent causes and potential drivers for change, our research aims to help the poor develop healthier eating habits. The widespread adoption of healthful food choices will not only benefit public health through decreases in obesity and obesity-related diseases but also reduce costs of government programs such as Medicaid and Medicare.

Methodology

Despite access to SNAP and school meal programs, many low-income households still favor low-cost, high-energy foods. We designed a 36-question survey and administered the questionnaire to individuals intercepted at fast-food chains and big-box retailers in low-income neighborhoods in the Dallas area. The survey explored why people, especially those in lowincome households, preferred fast food to home-prepared meals using fresh groceries and what measures could be taken to incentivize households to choose healthier foods. Also, we explored whether school meal programs had any influence on food choices and eating habits. In addition, we studied whether eating habits might be altered in response to other potential programs such as free community cooking classes, complimentary or discounted cookware and kitchenware sets for SNAP recipients, and community support of free meals.

Prospective participants included adults intercepted while dining in fast-food restaurants. The researcher approached the potential respondent and explained that she was doing research for a school project. After completion of the survey, the researcher explained the nature of the study to each participant and answered any questions raised. Due to differing situations associated with various locations, the researcher was flexible in approaching prospective participants either outside or inside the restaurants.

The method was reviewed by the Institutional Review Board at UT Dallas to ensure that the study was ethical and protected the rights of participants.

Results

The participants (n = 76) were distributed fairly between male (42.1 percent) and female (57.9 percent). Their ages ranged from younger than 20 years old to 70 years and older, with the largest age group being from 20 to 39 years old (55.2 percent). There were more people living alone (53 percent), including single and separated/divorced, than living with another, including either partner or spouse (47 percent). Their mean body mass index BMI was 26.7, which is an indication of being overweight.



Figure 1. Household income among survey participants and Dallas population.

In Figure 1, the comparison of household income among surveyed participants versus the Dallas population suggests that half of the respondents have household incomes less than \$20,000 (52.3 percent) compared to only 22.6 percent of the Dallas population.



Figure 2. Respondents' favorite healthful foods vs. frequent dine-out options.

Figure 2 displays the participants' favorite healthful food choices and their usual option when dining out. While 29.7 percent chose fruit and 28.4 percent chose salad as their favorite healthful food, none chose fruit and only 3.6 percent of the participants ate salad when they dined out. The most popular dining option was fast food (50 percent).

When asked about the barriers to changing eating habits, 45 percent of the participants indicated lack of time to prepare healthful food while 37 percent chose the cost of healthful food. Not knowing how to cook and confusion about diet plans each accounted for 8 percent of the responses. 43 percent of participants suggested free/ reduced cookware and groceries as a potential program. More participants chose cooking classes than community support (which includes free meal assistance) as a means of changing eating habits.



Figure 3. Regulation as a dependent variable.

As regulation is a continuous variable, we use the OLS regression approach. Number of observation = 76. R-squared = 0.3198. Adjusted R-squared = 0.2386.

Variable	Coef.	P>[t]	95% Conf. Interval
cons_healthy	.431	0.000	[0.241; 0.622]
bar_cost	.381	0.046	[0.006; 0.756]
snap	.552	0.015	[0.113; 0.991]
schoolmeal	481	0.027	[-0.904; -0.057]

Table 1. OLS regression analysis.

Table 1 examines the relationships among different factors (consumption of healthful food, barrier of cost, SNAP participation and school meal program participation) and the regulation psychometric measure, which is the respondent's self-perceived control over food choices. All p-values are smaller than 0.05, which indicates a significant

relationship between themselves and regulation. People who consumed healthful food felt significantly more in control of their food choices. People who listed cost as a barrier to eating more healthfully felt more in control of their food choices. Respondents on SNAP also felt more in control of their food choices. People whose children received school meals felt less in control of their food choices.

Variable	Coef.	P>[z]	95% Conf. Interval
bar_cost	5.718	0.001	[2.221; 9.215]
bar_time	6.613	0.001	[2.827; 10.400]
bar_cook	-5.127	0.020	[-9.450; 0.806]
regulation	2.589	0.016	[0.480; 4.699]

Table 2. Wish to change the eating habits as a dependent variable - logistic regression analysis.

As the wish is a discrete variable, we used logistic regression analysis. Number of observation = 71. LR chi2(9) = 67.90. Log likelihood = -14.68985. Pseudo R-squared = 0.6980.

Table 2 examines the relationships between different factors (barriers of cost, a lack of time and ability to cook, and regulation) and the wish to change current eating habits. All p-values are smaller than 0.05, which indicates the significant relationship between themselves and the wish to change. For respondents who wish to change their eating habits, cost and lack of time are significant barriers. Respondents who wish to change their eating habits feel more in control of their food choices, as shown by the positive and significant coefficient on the regulation psychometric measure. Interestingly, the coefficient on knowing how to cook as a barrier to changing food choices is negative and significant. An explanation for this is that people who do not know how to cook have a tougher time changing their eating habits and thus may answer that they do not wish to change their eating habits.

Conclusion

Survey data suggests that a lack of time and the cost of healthful foods are the main barriers for low-income households to improve the nutrition of their diets. Another significant barrier is not knowing how to cook, which is associated with a lack of motivation to change eating habits. While SNAP participants are more likely to be concerned about their current eating habits, those whose children participate in school meal programs are less likely to be concerned about their food choices. These findings encourage more supplemental programs to best assist the current SNAP and school meal programs. Potential ideas include free weekly community cooking classes to help motivate people to change their eating habits and learn how to cook more healthful food with less time. Other suggested programs include offering complimentary or discounted cookware and kitchenware along with fresh groceries using food stamps.

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Behavioral Economics and Health Economics: The Relationship among Patience, Saving Behavior and Health

In 2012, New York City passed a law prohibiting the sale of sugary drinks larger than 16 ounces. This law was based on the assumption that (in behavioral economics terms) some people failed to control their diet even when they were aware of the health risks related to high sugar intake. By limiting people's ability to consume, the law was intended to delay part of their desire for sugar intake, which would indirectly adjust their level of patience. This action of delaying instant gratification was defined as patience and had the potential to improve the public's health.

Other policies, however, have also been created with good intentions but have eventually failed to have a positive impact. For example, some cities in the United States (U.S.) have required fast-food restaurants to put nutritional labels on their food products. This knowledge of nutritional facts, though, can only deter people with a certain level of patience from eating the food. As with the case of sugary drinks, people have often overlooked the long-term health risk and have chosen instant gratification over long-term health.

- by Amy Thao Ngo

Introduction

These examples shed light on one of the reasons why some policies work and others don't. To be able to pass laws that can adjust impatient behavior and improve health, policymakers need to understand how impatience can cause inability to delay one's consumption. It is therefore important to study further the relationship between patience and health, in particular how patience influences one's diet behavior and how such behavior can affect the health of the public.

Literature Review and Background

A healthful diet is an important input to a person's wellbeing and longevity. Poor nutrition can lead to unhealthy conditions, including cardiovascular disease, cancer and obesity.¹ The costs of these conditions are high: for example, compared to people of normal weight, obese people will experience 14 percent to 25 percent more physician visits per year and six times more pharmacy dispenses for diabetes medication.² The long-noted importance of a healthful diet and its benefits for preventing cost-intensive health conditions has led economists and public health researchers to study the link between nutrition, overall health and behavior.

A popular hypothesis is that the ability to delay instant gratification directly affects how healthful a diet is. Anyone who has been on a diet can understand the difficulty of not eating dessert in order to achieve a better BMI and health long into the future. Previous literature has found a positive relationship between health status and patience.³ More recent work has also discovered that people with a lower discount rate (meaning more patient) have superior diet quality.⁴ We thus focus our research on testing a similar hypothesis: people who are more patient are likely to have a more healthful diet. We will use saving behavior as a proxy for level of patience and vegetable intake as a proxy for a healthful diet.

We used data collected from a low-income, minority neighborhood in South Dallas (hereafter, the Fair Park Neighborhood Study). The Fair Park Neighborhood Study was funded by the National Science Foundation and collected data from residents of the Fair Park Neighborhood from 2009 through 2012, to measure the impact of investment in the new Dallas Area Rapid Transit (DART) light rail line, which opened in the neighborhood in 2009.

Methods

How do we measure patience?

One method used to measure patience was to interpret participants' gambling choices. Volunteer participants came into a laboratory and received enough compensation to induce truthful behavior. We asked each participant a series of six questions; for each question, participants chose between receiving \$50 tomorrow or receiving a larger sum of money six months from tomorrow. The larger amounts of money increased with each consecutive question: \$51, \$55, \$60, \$70, \$100 or \$150, respectively. The more times a person chose to receive his payment six months later, the more patient he or she was judged to be. We divided participants into three categories. Participants who always chose to receive the money sooner or only delayed one time were put in category 1 (impatient people). Category 2 contained participants who chose the "later" payment in two or three decisions, and participants who selected the "later" payment in four or more of the six decisions were in category 3 (most patient people). The variable future indicated in which category a person belonged.

Even though the experimental method measured patience directly and accurately, the experiments were costly and difficult. An alternative method was to distribute surveys constructed with questions about saving/spending behavior, from which we could decipher the implied level of patience. In the Fair Park Neighborhood Study, we had one question asking participants about the most important period in planning their family's saving and spending. Participants were placed into three categories. Category 1 contained people who saved for the next few months (least patient). Category 2 contained people who saved for next year or the next few years (medium). Participants who saved for the next five to 10 years and longer than 10 years were put in category 3 (most patient). The variable save_period represented these categories.

Another question asked participants about their saving habits. Again, participants were divided into three categories. Category 1 had people whose answer was "Don't save – usually spend more than income." Category 2 had people who chose "Don't save – usually spend about as much as income" or "No regular plan – save whatever is left over at the end of the month." The last category contained people who actually planned their savings, indicated by "Save income of one family member, spend the other" or "Spend regular income, save other income," or "Save regularly by putting aside money each month." These answers measured how much effort participants put into planning ahead and were represented by the variable save_habit.

Theoretically, the more a person saves or plans to save, the more patient he or she is. Our question then is, how well do the surveys' results reflect one's true patience?

Hypothesis 1: Saving behavior reflects true levels of patience.

To test this hypothesis, we conducted multivariable ordered logit regressions on our experimental data (N = 174). Our dependent variable was future from the experimental studies and our main independent variables were, in turn, save_period and save_habits. In addition, the regressions also adjusted for age, gender, race, marital status, number of children under 18 years old and income level.

Descriptive Statistics

Experimental Data (N=174)		Survey Data (N=460)
Categorical Variables	Frequency	Frequency
save_period		
Least Patient	56%	59%
Medium	25%	25%
Most Patient	19%	16%
save_habits		
Least Patient	28%	26%
Medium	52%	52%
Most Patient	20%	21%
Income Level		
< \$20,000	73%	73%
[\$20,000, \$40,000]	17%	19%
> \$40,000	10%	8%
Gender		
Male	39%	38%
Female	61%	62%
Marital Status		
Married	18%	18%
Unmarried	82%	82%
Children Under 18		
Yes	55%	47%
No	45%	53%
Race (African American)		
Yes	94%	90%
No	6%	10%
Meals Prepared at Home	Each Week	
0		7%
1-5		54%
6-10		17%
11-15		10%
16-20		6%
20+		6%
Continuous Variable	Mean (SD)	Mean (SD)
Age	44 (13)	44 (15)

Table 1.

Results

As depicted in Table 1, our sample was rather homogeneous. Participants were primarily low-income (73 percent with annual income lower than \$20,000), unmarried (82 percent), African American (94 percent) and women (61 percent). The average age was 43 years and about half of the participants had children. This homogeneity had the potential to affect our regression results. For instance, we could argue that most people did not have enough money to save with such low income. The distribution of saving variables displayed such concerns: 56 percent were in Category 1 (only save for the next few months), and 51 percent were in Category 2 (little financial planning but not in financial trouble). Neighborhoods with these demographic traits, however, had been the main targets of governmental development grants. Studying these neighborhoods is important in assessing and improving development actions.

Regression Results for Experimental Data

	Model 1 (Save_Period)	Model 2 (Save_Habits)
save_ period	0.244 **	
	0.034	
save_habit		0.267 *
		0.06
Age	0.005	0.009
	0.477	0.235
Income Level	-0.113	-0.133
	0.45	0.377
Gender (Male)	-0.605 ***	-0.513 **
	0.003	0.012
Married or Not	0.285	0.189
	0.258	0.457
Having Children Under 18 Years Old	-0.184	-0.124
	0.372	0.543
Race (Black)	0.897 *	0.928 *
	0.062	0.054
Ν	174	174

*p < 0.10 **p < 0.05 ***p < 0.01

Table 2.

Table 2 presents the regression results for both models and Table 3 reports the marginal effects for save_period and save_habits. Marginal effects might be interpreted as the percentage change in likelihood of being in a particular category from a marginal change in the relevant independent variable. The marginal effect for model 1 (with save_period) indicates that the likelihood of being impatient decreases by 8.8 percent, and the likelihood of being patient Marginal Effects for Saving Behavior According to Level of Patience

Categories for Future	save_period	save_habits
Impatient	-0.088 **	-0.097 *
Medium	0.053 **	0.058 *
Patient	0.036 **	0.039 *

p < 0.10 p < 0.05 p < 0.01

Table 3.

increases by 3.6 percent for each one category step-up in save_period. Similarly, in model 2 (with save_habits), the likelihood of being impatient decreases by 9.7 percent and the likelihood of being patient increases by 3.9 percent for each one category increase in save_habits.

Do we have a better diet when we are more patient?

With these findings, we confidently used saving behaviors as proxies for level of patience in testing the relationship between patience and diet. A healthful diet is composed of a balance of carbs, fat, protein, and vitamins and minerals. Vegetable intake, represented in our data by the variable hVeg_nofries_ad, has been found to be one of the most important indications of a healthful diet.⁵ This variable is calculated from responses to the NCI Multifactor Screener (NCI 2000). The Multifactor Screener asks a series of 16 questions regarding the type and frequency of food consumption. Based on responses to these questions and the participants' gender and age, different measures of nutritional intake were calculated, including pyramid servings of fruits and vegetables consumed (excluding french fries) and grams of fiber consumed.

Hypothesis 2: People who are more patient consume more vegetables.

We conducted OLS linear regressions using hVeg_nofries_ad as our dependent variable on survey data (N = 441). As presented in Table 1, both experimental data and survey data shared similar demographic characteristics. Our main independent variables were, in turn, save_period and save_habits. In addition to controlling for all the variables included in the previous regressions, we introduced "number of meals prepared at home per week" to our models.

Results

Regression results are presented in Table 4. Among the demographic controls, we found age and gender to be weakly related to vegetable intake. We also confirmed our assumption about meals cooked at home. In both models, it is statistically significant that more meals cooked at home had a positive effect on the level of vegetable intake.

	Model 1 (save_period)	Model 2 (save_habits)
save_ period	0.12 *	
	0.051	
save_habit		0.187 ***
		0.007
Age	-0.01 ***	-0.009 **
	0.004	0.015
Income Level	-0.028	-0.041
	0.722	0.596
Meals Cooked at Home per Week	0.124 ***	0.129 ***
	0.001	0.000
Gender (Male)	0.161 *	0.169 *
	0.103	0.085
Married	0.081	0.0165
	0.511	0.895
Having Children Under 18 Years Old	-0.014 *	0.027 *
	0.889	0.793
Race (Black)	0.167	0.164
	0.316	0.321
Constant	1.41 ***	1.162 ***
	0.000	0.000
Ν	441	441

*p < 0.10 **p < 0.05 ***p < 0.01

Table 4.

Both save_period and save_habit coefficients were statistically significant (p-value equals 0.063 and 0.01, respectively). In the first model, when participants moved up one category in saving period (more patient), they consumed 11.3 percent more vegetables. In the second model, when participants moved up one category in saving habit (by planning ahead and being more patient), they consumed 17.3 percent more vegetables, *ceteris paribus*.

Conclusions

There are essentially two important results from our research. We have found a strong correlation between levels of patience measured in a laboratory environment and that found in some saving behaviors. This confirms our belief that saving behaviors are appropriate proxies for patience. Our finding contributes to the confidence in measuring patience using the survey methods in place of conducting costly experiments in future works. We also confirm the assumption that the number of meals cooked at home is positively related to level of vegetable intake. Most importantly, even though our participants are from a low-income neighborhood and do not have much to save, we still find a positive correlation between their level of patience (proxied by saving behaviors) and the level of vegetable intake.

A growing body of behavioral economists believe that "the greatest potential for improving the health of the American people ... is to be found in what people do and don't do to and for themselves."⁵ Impatient behavior not only affects long-term health, but also can disqualify people from health insurance rebates, which can negatively affect their financial situation. "Nudges" like taxation on sugary products can guide people to achieve better health. Understanding the different factors that contribute to one's diet helps construct better policies and "nudges" that not only educate the population on making better financial decisions but also improve health conditions.

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Determination of the Role of Reactive Oxygen Species in a Cell Culture Model of Neurodegenerative Tauopathy

> Tauopathies are neurodegenerative diseases characterized by the aggregation of the microtubule-associated protein tau. Among all forms of neurodegenerative diseases, Alzheimer's disease (AD), which is associated with progressive memory loss, is the most common and challenging. Currently, AD is the fourth leading cause of death and more than 5 million people have been diagnosed with AD in the United States. Two forms of protein aggregates are involved in AD: extracellular aggregates made of amyloid- β protein (A β) and intracellular aggregates of tau protein, defined as neurofibrillary tangles (NFTs).

Mitochondria play crucial roles in cellular metabolism as the "power house of the cell." As the major sites of reactive oxygen species (ROS) generation, mitochondria are also the primary targets of ROS. Oxidative stress is caused by the imbalance between prooxidants and antioxidants.¹ ROS, such as hydrogen peroxide (H_2O_2) and free radical superoxide anion (O_2-) , are highly reactive chemicals with unpaired valence shell electrons. When compared with other organs, the brain is more susceptible to oxidative stress because of its relatively high metabolic rate and low antioxidant concentration.²

— by Xiao Tan



Introduction

Our laboratory has developed a cell culture model of neurodegenerative tauopathy using a six amino acid-long sequence (VQIVYK) located in the third microtubule-binding repeat of tau rendered cell permeable by the addition of a polyarginine tag (VQIVYK-R₉; T-peptide).³ We have observed that T-peptide can form AD-like PHFs in vitro and have found that T-peptide is highly neurotoxic.⁴ Cell death and a decrease in mitochondrial membrane potential were observed within 24 hours after treating cells with T-peptide.⁵ Core peptide (VQIVYK) and a peptide with a mutation in the six amino acid sequence, K-peptide (VQIVKK-R₉), were also used to determine the mechanism of T-peptideinduced neuronal cell death. We have found that neither K-peptide nor core peptide is neurotoxic, which indicates that neurotoxicity is sequence-dependent and is enhanced by cell permeability.⁶ The molecular mechanism by which T-peptide induces cell death and mitochondrial dysfunction is not known. In this study, I found that T-peptide can induce ROS accumulation by measuring intracellular ROS levels in T-peptide-treated mouse hippocampal neuroblastoma cells (HT-22). Also, I found that ROS accumulation is an early event in T-peptide-induced neuronal cells' death by examining the time course of the induction of ROS in T-peptide-treated HT-22 cells.

Materials and Methods

HT-22 cells, mouse hippocampal neuronal cell line, were maintained in 60-mm-diameter polyethyleneimine-coated dishes in DMEM supplemented with 10% FBS, 50 U/ml penicillin and 50 μ g/ml streptomycin. HT-22 cells were incubated in a humidified incubator with 7.5% CO₂ at 37°C. Experiments were performed in cells that had been cultured for one or two days to reach about 70 percent confluency.

Peptides were provided by Dr. Warren J. Goux, Department of Chemistry, The University of Texas at Dallas. Images were taken using a Nikon Eclipse TE300 fluorescence microscope. To avoid photooxidation and photobleaching, 10 images were taken for each well or coverslip. Images were taken in a counterclockwise order to minimize photooxidation. At least 300 cells were imaged from each well or coverslip. All images were taken at 400X magnification. The fluorescence intensity/cell was quantified using Image J (http://rsbweb.nih.gov/ij).

The data were analyzed by using unpaired student t-test. Unless otherwise mentioned, data are expressed as mean \pm standard error of mean (SEOM). A p-value of <0.05 was considered significant.

The intracellular ROS levels were determined by using CM-H₂DCFDA, a cell-permeable dye whose two acetate groups can be cleaved by intracellular esterases to yield DCFH. Oxidative of DCFH by ROS within the cells can convert the nonfluorescent DCFH to the fluorescent molecule DCF. This allows us to directly detect intracellular ROS levels by measuring fluorescence intensities using a fluorescent microscope.

The mitochondrial membrane potential was measured by using Rhodamine 123 (Rh123). Rh123 is a fluorescent cationic dye that accumulates in the mitochondria as a function of the mitochondrial membrane potential.

Results

In our laboratory, we have found that T-peptide is highly neurotoxic. In contrast, core peptide and K-peptide were not found to be cytotoxic as judged by "Live/Dead Assays".⁷ To determine if ROS accumulation might play a role in T-peptide-induced neuronal cell death, we measured the intracellular ROS levels in HT-22 cells after treatment with K-, T- or core-peptide.





The intracellular ROS levels were significantly higher in T-peptide-treated cells versus K-peptide or core-peptidetreated HT-22 cells (Figure 1a). Furthermore, significant cell death was observed in HT-22 cells after treatment of T-peptide, whereas there was little cell death in K- or core-peptide-treated cells (Figure 1b). Also, nuclear fragmentation was not observed in T-peptide-treated HT-22 cells, which is in agreement with our previously published results (Figure 1b).



Figure 1b. The effects of T-peptide, K-peptide and Core-peptide on Intracellular ROS Levels. a) Quantitative analysis of intracellular ROS levels. Values are expressed as mean DCF fluorescence/cell \pm SEOM. *p<5.0E-04; **p<5.0E-06; ***p<5.0E-09. b) Representative images of DCF fluorescence in peptide-treated HT-22 cells. The arrows indicate a dead or dying cell. Scale bar = 30 µm.

We also measured the intracellular ROS levels in HT-22 cells treated with various concentrations of T-peptide for three hours. We have found that the intracellular ROS levels in T-peptide-treated HT-22 cells increased in a concentration-dependent manner (Figure 2).



Figure 2. Intracellular ROS levels in HT-22 Cells treated with various concentrations of T-peptide. *p<0.005; **p<5.0E-04; ***p<5.0E-05 with respect to control cells.

In an attempt to determine the role of ROS accumulation in T-peptide-induced neuronal cell death, we examined the time course of the induction of ROS in T-peptide-treated HT-22 cells. We measured the intracellular ROS levels in HT-22 cells treated with T-peptide for one hour, two hours, three hours, six hours or 24 hours. When compared with cells treated with T-peptide for one hour, the cells after two hours of treatment with T-peptide increased significantly (Figure 3). The intracellular ROS levels remained relatively constant after three hours of treatment and dropped dramatically after 24 hours of treatment with T-peptide (Figure 3). There was no significant difference in control cells after 1 hour and 2 hours (Figure 3). Thus, the finding was that ROS accumulation is an early event in T-peptideinduced neuronal cell death.



Figure 3. ROS accumulation is an early event in T-peptideinduced neuronal cell death. *p<0.05; **p<0.0005 compared with control cells.

To determine if glutathione can protect HT-22 cells against increased production of intracellular ROS induced by T-peptide, we compared the intracellular ROS levels in HT-22 cells treated with T-peptide alone versus T-peptide together with glutathione. For this experiment, cells were grown on glass coverslips. A significant increase in intracellular ROS levels was observed in T-peptide-treated HT-22 cells (Figure 4). The intracellular ROS levels in cells treated with T-peptide together with glutathione were similar to control cells, indicating that glutathione can protect cells against the increased ROS production induced by T-peptide (Figure 4).



Figure 4. The effect of glutathione in reducing intracellular ROS levels generated by T-peptide. **p*<5.0*E*-03; ***p*<5.0*E*-04.

Mitochondrial membrane potential is an important parameter of mitochondrial efficiency. In an attempt to determine the role of ROS accumulation in mitochondrial dysfunction, we measured the mitochondrial membrane potential in HT-22 cells treated with T-peptide alone and T-peptide together with glutathione. Valinomycin, used to dissipate the mitochondrial membrane potential, caused a significant reduction in mitochondrial membrane potential in HT-22 cells (Figure 5b). We found that glutathione alone did not alter the mitochondrial membrane potential (Figure 5a). We also found that T-peptide could reduce mitochondrial membrane potential (Figure 5a; 5b). This agrees with previous data from our lab using JC-1.8 The mitochondrial membrane potential in cells treated with T-peptide together with glutathione was similar to the control cells, which indicates that glutathione can rescue the T-peptide-induced decrease in mitochondrial membrane potential (Figure 5a).



Figure 5a.



Figure 5b. The effects of T-peptide and glutathione on the mitochondrial membrane potential in HT-22 Cells a). Quantitative analysis of mitochondrial membrane potential by using Rh123. Data was expressed as mean Rh123 fluorescence/cell \pm SEOM. *p<0.05; **p< 0.005 with respect to control cells. b) Representative fluorescent images of Rh123 fluorescence in HT-22 cells. Scale bar = 30 µm.

Discussion

ROS accumulation has attracted great interest in the study of neurodegenerative tauopathy. It has been suggested that oxidative stress plays an important role in neurodegenerative diseases, such as AD, Parkinson's disease and Huntington's disease.

In this work, we demonstrated that intracellular ROS accumulation plays an important role in T-peptide-induced neuronal cell death by comparing intracellular ROS levels in T-, K- and core-peptide-treated HT-22 cells. We have found that the intracellular ROS levels in T-peptide-treated HT-22 cells were much higher when compared with K- and core-peptide-treated cells. Also, K-peptide and core-peptide are not cytotoxic, which suggests that ROS accumulation contributes to the T-peptide-induced neuronal cell death.

Our data indicate that intracellular ROS accumulation is an early event in T-peptide-induced neuronal cell death. We observed a significant increase in intracellular ROS levels atearly-stage after treatment with T-peptide. The intracellular ROS levels dropped dramatically at late-stage in our cell culture model, which suggests that ROS accumulation may happen prior to other cytopathology in AD. The work presented above also shows that T-peptideinduced intracellular ROS levels increase in T-peptideinduced neuronal cells in a dose-dependent manner. Cell death was not observed in cells treated with T-peptide at low concentration. The concentration-dependent manner may provide the evidence that the treatment of exogenous T-peptide can increase the intracellular ROS to a toxic level, which can kill the cells or trigger other events that can initiate cell death, such as elevating intracellular Ca²+ levels.

It has been suggested by others that mitochondria play a crucial role in age-related neurodegenerative tauopathies. Many lines of evidence show that mitochondrial DNA mutation and production of excess intracellular ROS contribute to neurodegenerative diseases. It has been suggested by others that ROS is generated at high mitochondrial membrane potential. However, we observed a decrease in mitochondrial membrane potential in T-peptide-treated HT-22 cells. We proposed that ROS accumulation happens before mitochondrial membrane potential decrease. To confirm this hypothesis, we compared the mitochondrial membrane potential in cells treated with T-peptide alone versus T-peptide together with glutathione. The mitochondrial membrane potential in cells treated with T-peptide together with glutathione was similar to the control cells, which indicates ROS accumulation may be the primary event that leads to mitochondrial membrane potential reduction. It has also been suggested by others that mitochondrial membrane potential decrease is a consequence of ROS accumulation.⁸

The decline in intracellular glutathione concentration and increase of oxidative stress has been suggested to precede the signaling events associated in apoptotic cell death. Mitochondria are involved in apoptotic cell death by alternating the redox potential. It has been suggested by others that apoptotic cell death may be involved in neurodegenerative tauopathies. However, nuclear fragmentation, a hallmark feature of apoptosis, was not observed in T-peptide-treated neuronal cells, which indicates that the T-peptide-induced neuronal cell death is not apoptotic. In our previous study, we have found that leupeptin, a lysosomal protease inhibitor, can rescue neuronal cells from T-peptide, which indicates that lysosomes are involved in T-peptide-induced neuronal cell death. It has been suggested that the ROS accumulation induced by intracellular amyloid aggregates can lead to lipid peroxidation, which can lead to lysosomal membrane leakage. The release of lysosomal hydrolases into the cytosol may be a key event that leads to neuronal cell death. We will further examine the role of ROS accumulation in lysosomal leakage by monitoring lysosomal membrane disruption in cells treated with T-peptide alone versus T-peptide together with glutathione using Acridine Orange.

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SIMULATION OF BACKGROUND SOURCES FOR THE FUTURE UNDERGROUND DARK MATTER EXPERIMENT EURECA

It is well established that visible matter consisting of protons, electrons and neutrons accounts for only one-sixth of the total mass of the universe. Dark matter's presence is inferred from its gravitational effect on rotational periods of stars about their galaxy's core, collisions of clusters of galaxies, and gravitational lensing effects.

A candidate for dark matter is the Weakly Interacting Massive Particle (WIMP), a class of hypothetical particles that are not affected by the strong nuclear force or electromagnetic fields. The absence of these interactions renders WIMPs practically invisible. Dark matter remains one of the biggest unsolved mysteries in astrophysics; therefore, direct experimental evidence of a dark matter particle candidate would significantly contribute to the progress of modern science.

— by Thong Q. Nguyen



Introduction

The EURECA (European Underground Rare Event Calorimeter Array) project is designed to search for WIMPs by detecting the elastic scattering of WIMPs by atomic nuclei with an unprecedented sensitivity. The EURECA experiment is designed to be installed at the Deep Observatory for Multidisciplinary Underground Science (DOMUS), a planned extension of the Modane Underground Laboratory, the deepest underground laboratory in Europe. EURECA employs low-temperature cryogenic technology to detect phonon and either ionization or scintillation from Germanium or CaWO₄ crystals, respectively, with one ton of target mass. Phonon is a unit of vibrational energy that arises from oscillating atoms within a crystal. Scintillation is a flash of light produced in certain materials when they absorb ionizing radiation. Radioactivity and cosmic rays would impose disabling backgrounds on the detector of EURECA's sensitivity. External and internal shielding reduces the rate from these sources, and a veto system tags residual background.

This goal of this research was to provide a more accurate implementation of the optical sensor simulation and to evaluate background signal rates from ambient radiations. GEANT4, a toolkit for the simulation of the passage of particles through matter, was used. Decays of Cobalt-60 (⁶⁰Co) and Potassium-40 (⁴⁰K), which produce gamma rays and electrons, were simulated inside the components of EURECA. If these electrons produce a high signal rate in the tank's sensors, they could "flood" the veto system with nuisance signals, making veto insensitive to the backgrounds of concern.

The EURECA Experiment

The EURECA project is a joint effort among scientists from the EDELWEISS and CRESST collaborations. EDELWEISS and CRESST are underground cryogenic experiments employing the above described crystals at very low temperatures. The current concept of the EURECA project consists of a shield of a large water tank containing the cryostat housing the detectors. EURECA will be installed at the Modane Underground Laboratory—located between France and Italy to minimize the background of cosmic muons produced in collisions between cosmic rays and the Earth's atmosphere. EURECA employs two types of cryogenic detectors: the Germanium bolometers, developed by EDELWEISS, that measure heat and ionization signals, and CaWO₄ scintillating bolometers, developed by CRESST, that measure light and heat signals.¹ The cryostat housing these detectors is shielded by a cylindrical water tank of 8 meters in diameter and 12 meters in height. The thickness of the stainless steel tank walls is 50 centimeters. The principal component of the cryostat is copper. The water tank and the cryogenic unit are placed inside the DOMUS laboratory, which is lined with 1 meter of concrete.²



*Figure 1. Position of the water tank within the cross section of the DOMUS laboratory, approximately to scale.*³

Background Sources

The EURECA project aims to reach a sensitivity for WIMP-proton scattering corresponding to a cross section of about 10⁴⁷ cm², more than 20 orders of magnitude smaller than typical nuclear scattering processes, with a residual total background of less than one event/year per 1,000 kilograms of detector mass.⁴ Reaching such a level of sensitivity requires selecting high radiopurity materials and evaluating background suppression through simulation work. The background sources in EURECA include nuclear and electron recoils, induced by cosmogenic radiation and ambient radiation. This research project focused on evaluating radioactive backgrounds coming from the components of the water tank.

Radioactive decay products include alpha particles, electrons, positrons, gamma rays and neutrons.⁵ Gamma rays coming from outside are suppressed using passive shielding, including the steel tank, water and the copper cryostat. Furthermore, gamma rays still reaching the

crystals generate electron recoils in the detector, which can be discriminated from nuclear recoils expected from WIMPs by the simultaneous measurement of phonons and ionization or scintillation.⁶ Despite these enormous suppression mechanisms, detailed simulations are required to assure that less than a single gamma ray per year may be misinterpreted as a WIMP scattering in the 1-ton target.

In this paper, the following components of the water tank are examined as sources of ambient radiation: the stainless steel tank, the copper cryostat and the photomultiplier tubes. Radioactivity from ⁶⁰Co and ⁴⁰K in these components' materials are evaluated based on their decay products and the physics process inside the water tank.

Physics Processes

1. Beta Decay

Beta decay is a radioactive decay in which a beta particle, which can be either an electron or a positron, is emitted from an atomic nucleus. In the water tank's shielding and detector components, decays of ⁶⁰Co and ⁴⁰K emit electrons that can ultimately deposit energy in the detector. It is therefore necessary to determine the rate of background electrons emitted from the water tank's components. If these electrons produce a high signal rate in the tank's sensors, they could "flood" the veto system with nuisance signals, making veto insensitive to the backgrounds of concern.

2. Cherenkov Effect

The Cherenkov effect, named after the Russian physicist Pavel Cherenkov, occurs when a charged particle moves faster than light in a material medium. The charged particle polarizes the surrounding molecules that rapidly return to their ground state, emitting coherent light called Cherenkov radiation.⁷ The wavelength of Cherenkov radiation ranges from 400 to 700 nanometers, corresponding to the ultraviolet and visible ranges of the electromagnetic spectrum. The light is detectable using an optical sensor called a photomultiplier. The threshold kinetic energy of an electron in water for the emission of Cherenkov radiation is 0.260 MeV.⁸

Beta decays of ⁶⁰Co emit electrons with energy of 0.31 MeV (99.88 percent) and 1.48 MeV (0.12 percent). The predominant decay of ⁴⁰K emits an electron with a kinetic energy of 1.31 MeV. All these decays exceed the Cherenkov threshold and produce photons.

3. Photon Detection

Photomultiplier tubes (PMTs) are photon detectors that convert photons to electrical signals. Photomultiplier tubes amplify the primary photoelectrons produced at their photocathode factors as large as 100 million times. When the rate is sufficiently low that signals do not overlap, PMTs provide a way to detect individual photons.



Figure 2. Photomultiplier tube. Multiple dynode stages amplify the primary photoelectrons produced by the incident light.⁹

The EURECA's water tank is equipped with 72 PMTs distributed in six rows inside the tank's wall to detect Cherenkov radiation generated by electrons in water.

Simulation Methods

The models of the water tank and its components were implemented within the GEANT4 simulation toolkit. Beta decays of ⁶⁰Co and ⁴⁰K were simulated inside each component of the tank, i.e., the emitted beta particles and gamma rays were started and tracked, to evaluate PMT signals from background electrons from these sources.

I implemented a new configuration of PMTs that included a reflective layer at the back of the PMT window and a rubber cover wrapping the cylindrical body of the PMT, and examined the difference in rates of Cherenkov photons detected by the two models of PMTs. The new implementation, which is a more accurate model of the actual PMT that will be installed for the EURECA experiment, was applied in all simulations performed in this paper.

Each simulation was run with 10⁵ events of beta decays of ⁶⁰Co and ⁴⁰K distributed randomly in each component of the water tank: the stainless steel, the copper shield and the PMTs. With the recorded information of the number of PMTs that were hit by Cherenkov photons and the number of Cherenkov photons triggering one PMT, signal frequencies of these PMTs can be determined.



Figure 3. A-Steel Tank, B-Copper Shield, C-Crystals, D-PMT. GEANT4 implementation of the water tank and its components. The crystal detector array is installed in the cryostat at the center of the tank, surrounded by the copper shield. The steel wall of the tank is equipped with 72 PMTs.



Figure 4a. A-Rubber Cover, B-Reflective Layer. New implementation of the PMT (on the right). A reflective layer at the back of the PMT window and a rubber cover wrapping the cylindrical body of the PMT are installed.



Figure 4b. The PMT used in the EURECA experiment.

Results and Discussion

The PMTs are set to be triggered when at least one single photoelectron is produced through a Cherenkov photon absorbed in the PMTs. An event is counted when at least one PMT is triggered.

1. Comparison of the two implementations of PMTs



Figure 5. Rates of events in which at least one PMT is triggered by one, two, three, etc., photoelectron(s) with two implementations of PMTs.

With the new implementation, the PMTs are triggered with a lower frequency. The rate of unwanted events from radioactive decays in which at least one PMT is hit by two or three photoelectrons is reduced by half when the rubber cover and reflective layer are appended to the PMTs.

2. PMT signals from beta-decays of ^{60}Co and ^{40}K in the water tank's components



Figure 6. Rates of PMT signals from beta-decays of ⁶⁰Co started in the copper shield and the steel tank.



Figure 7. Rates of PMT signals from beta-decays of ⁴⁰K started in the PMTs, copper shield and steel tank.

The rate of PMT signals from decays of ⁶⁰Co and ⁴⁰K in the copper shield is very low compared to that in the steel tank. This results from the lower radioactivity of ⁶⁰Co and ⁴⁰K in copper, as indicated in Table 1, and the difference between distances of copper shield and steel tank to the PMTs. The PMTs are placed 30 centimeters from the wall of the steel tank, while the copper shield is installed near the center of the water tank, 2.7 meters from the nearest PMTs. The PMTs themselves are sources of beta-decays of ⁴⁰K, which emit background electrons and generate Cherenkov photons in the water tank. These Cherenkov photons from decays of ⁴⁰K in PMTs are produced at very close distances to the PMTs, resulting in the highest rates of undesirable background PMT triggering.

	Steel Tank	Copper Shield	PMTs
60 Co	1832.3 Bq	0.543 Bq	0
⁴⁰ K	488.6 Bq	3.8 Bq	151.2 Bq

Table 1. Radioactivity (in decays per second, or Bq) of ⁶⁰Co and ⁴⁰K as implemented in the simulations in different components of the water tank. The values are taken from a radiopurity database and compiled according to the mass of each component.¹⁰

Conclusions

The signal rates from ambient radiation backgrounds are low enough not to create any significant dead time for the WIMP search; for example, when the threshold to trigger the PMTs is set at 3 photoelectrons, the highest signal rate from beta decays of ⁴⁰K started in the PMTs is 0.915 events per second, with the dead time of 10 nanoseconds for one pulse in the PMT, resulting in a total dead time of 7.9×10^{-4} second per day, or 9.2×10^{-7} percent.

Detailed geometry for the PMTs changes the resulting signals rate, as Figure 5 has shown. However, further optimization of the PMT implementation is necessary. The reflective layer and rubber cover of the PMTs are currently set with simplistic values, which are 100 percent and 0 percent reflective, respectively. Experimental data of the reflectivity of the materials corresponding to different wavelengths may help to improve the accuracy of the PMT implementation.

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About the creative contributors



Clarissa Pollack is a senior arts and performance major who grew up in Montana. She enjoys horseback riding, exploring, and making sushi. Clarissa also performs as a violinist, vocalist and dancer. This spring, Clarissa is in Marburg, Germany, where she is studying conflict resolution, art history and humanity. She is an AES National Merit Scholar and recently, the Boys and Girls Club of Richardson, Texas, named Clarissa Volunteer of the Year.



Nehal Mubarak, a junior creative writing major, came to the United States from Jeddah, Saudi Arabia, in 2001. At the age of eight, medicine fascinated her and she aspired to be a doctor to fulfill her dream of helping people. Determined to improve her English, she began writing poetry soon after settling in the United States. After beginning her freshman year at The University of Texas at Dallas, she realized that, while she embraced the idea of / being a doctor, what she truly wanted to do was write. Nehal decided to forgo the pre-med program and instead pursue a bachelor's degree in creative writing, saying that she has never regretted that life-changing decision. She is inspired by many writers of literary fiction, including Joyce Carol Oates, Fyodor Dostoyevsky and William Faulkner. Nehal claims, "Most days I'm holed up in the sanctuary of my room (and my mind), succumbing to the whims of the Muse."



is a junior arts and technology student from Portland, Oregon. As a proud Bryce Jordan Creative and Performing Arts scholar, she aspires to become a professional graphic artist and illustrator. Currently, she serves as an officer for the Student Game Developer Alliance, and has produced artwork for a variety of games and student animations. Her lighter works appear around the UT Dallas campus in the form of club posters and offbeat cartoons. She can most often be found huddled over a computer and drawing tablet, and would greatly appreciate it if someone brought her some more caffeine.





, of Plano, Texas, completed a bachelor's degree in arts and technology with a focus on game production in December 2013, graduating summa cum laude. Introduced to video games at a young age, Kelly has always been fascinated with game development as a hobby and was ecstatic when she discovered the arts and technology degree program at UT Dallas. Having worked in law and engineering prior to her studies, Kelly's professional background has helped her understand the challenges of consolidating talent across diverse teams. With nearly a decade of practice assisting small businesses and a working knowledge of project management, she is excited to use her experience and passion for gaming to support others as a producer. Kelly's recent work includes leading a team of 15 graduate and undergraduate students in development of The Fast and the Fjorious game at UT Dallas. Additionally, she currently volunteers for Tiny Thumbs Arcade, a local art show collective for pop-up arcades, which promotes awareness for the indie game scene.

Inseph Castillo is a freshman arts and technology major from Houston, Texas. As a child, he was enthralled by fiction writing and quickly attempted to write his own stories involving heroes saving the world. Although he originally planned a career in biomedicine, Joseph changed course during high school and chose to pursue his dream of creating video games. Currently, Joseph aspires to become a game writer but states that "becoming any sort of writer would be pretty great, too."



Desirée Alicea-Aponte is a Puerto Rican from Houston, Texas. She is currently a freshman majoring in arts and technology at UT Dallas, with aspirations of becoming a storyboard artist and animator. Currently, Desi is working on publishing several independent comics with her collaborator, Joseph Castillo, with whom she created "Projections and Reflections." Typically, Desi can be found drawing in her sketchb o o k, enjoying a film from Hollywood's Golden Age, or browsing at the local comic book store.



eg Slagel, a self-described "pioneer," graduated from UT Dallas in the spring of 2013 with a degree in arts and technology. He was a founding member and first president of the Animation Guild at UT Dallas, as well as one of the first members of the Undergraduate Dean's Advisory Council. Greg directed one of UT Dallas' largest animated film productions, Bird in a Cage, and was the first project coordinator in the two-semester Animation Production class. In his senior year, he was awarded the Presidential Achievement Scholarship, and upon graduating cum laude, received major honors for his senior thesis film, Bird in a Cage.



Change

I find liberation in painting. The paint is out of my control if I allow it to be, and this paradox is what draws me to create paintings. Unfortunately, the temptations to revise, clarify and define my work in order to "achieve" something often lead me to make changes that I later regret. When I "correct" my works, they grow stale and rigid along my quest for perfection. This piece is different. It was never polished. I created it with curiosity, the leftover acrylic paint in my brush and a scrap of textured cardstock. With a single swift swish of the brush, I watched in awe as the pigments changed. Paint sometimes does amazing things that are far more beautiful than the artist could plan.

This painting, titled "Change," is an honest story of itself. I want to let the paint show the viewer how this change happened. Had I left the paintbrush at a single angle, it would have created a bluish muddle. Had I followed a straight trajectory, the rhythmic qualities of this sine wave would be lost. Instead, I twisted my brush and let my arm dance, and that allowed each color to shine! I love to see these bold primary colors coexisting in such harmony; they come together gracefully and preserve the integrity of each changing hue. The creation of this work taught me that I want to be free to display all of my colors the way this brushstroke revealed the inner prism of color hidden within the paintbrush.

- by Clarissa Pollack

We are the negatives In the dark room Glowing in the red That is our anger Anxious to be released From the confines Of this gripping Reality We are the black and whites Dipped and left to dry And still we remain Undeveloped

— by Nehal Mubarak



Altim

Poetry is expression. It allows you to say things that you would never dare to speak aloud, things that you are sometimes surprised you were thinking at all. Poetry allows the writer to know himself, so that he may discover the extent of his creative imagination and work toward pushing its limitations. Poetry is pain without having to be pain, a medium that can bring isolation and joy together without feeling the need to make excuses for itself. It drains the senses, only to fill them with something else entirely, and there is always something else, clawing at the corner of the mind and demanding to be heard.

I write poetry mostly because I don't have a choice. When that hunger to transfer my heart onto paper comes, I am completely and utterly enslaved to its will. I do not pretend to understand it, nor do I believe that I ever will, but I love it just the same. Poetry is what gives me voice, although I am just the messenger. The words, they often tell me, like to speak for themselves.

It's always hard for a writer to hear the question, "What inspired you to write this?" primarily because we often don't know. Would it be too simple to say that poetry is my inspiration? Too often I don't see it coming, the onslaught of thoughts barreling through my mind and frantically waiting to be written. But, if I were to put a name to it, I would say that life itself influences and inspires me. The amputee waiting to cross the road at a busy intersection while the rest of the world drives by or the child walking through the aisle of a grocery store, tears glistening in her eyes as she follows her inattentive mother—small details that we pass every day but never stop to consider—those are the moments I take advantage of. Suddenly a word is born, a phrase constructed. And the rest ... well, the rest is poetry.

This particular poem is a metaphor that takes into consideration the role we play as human beings in today's world, how influenced we are by what goes on around us, and how important it is for everything (and everyone) to be politically correct.

2D Concept Art of Villages 1 2D Concept Art of Level Progression 2D Concept Art of Village Houses 3 2D Concept Art of Thor's Hammer 4 2D Concept Art of Characters (5) 3D Render of Character Models 6 3D Render of Character Models (Textured) 3D Render of Game (Forest Level) 8 3D Render of Game (Thunder Plains Level) 9

45

9



The Fast and the Fjorious was a team-based project developed in the fall 2013 session of Game Production Lab at The University of Texas at Dallas. Game Production Lab is an industry simulation course in which teams of students collaborate to create a functional game demo within one academic semester. The fall 2013 session of Game Production Lab was taught by Dr. Monica Evans and supervised by Kyle Kondas and Skylar Rudin.

The Fast and the Fjorious project is a 3D, two-versus-two racing game that features sprinting cartoon Vikings on a mad dash to obtain Thor's glorious hammer, which has fallen from the sky a good distance from the player's home village with dense, harsh forest in the way. In the game, there are two teams, which consist of two players per team. The teams race through fjords, over glaciers and across volcanic environments. The goal is to reach the hammer before the other team.

The game development team was composed of extremely talented artists, a skilled programmer, a passionate producer and a committed quality assurance (QA) team. Overall, the combined dedication of 15 students brought the Vikings to life, from previsualized concept to a playable 3D game.

- by Cara Curley and Kelly Padgett

The Fast and the Fjorious Development Team

Creative Director Cara Curley

Producer Kelly Padgett

Programming/ Networking Zach Hiscocks

Level Design Carrie Crossley Richard Wirth Leighton Luckey

Art Director Tashkeel Shah

3D Modeling/Texturing Artists Lorraine Bett Ann Parent Kristine Dickson Tashkeel Shah Emily Pich Cara Curley

Game Design Leighton Luckey Tony Schuler

Rigging Shona Gillard

Animation Alex Rothenberg

Sound Design Chris Miller

Special thanks to Stephan Martiniere, the Game Production Lab QA team, and the Arts and Technology Department at The University of Texas at Dallas.





Projections Reflections

"Don't judge a book by its cover." Many of us heard these words when we were quite young and assumed the popular phrase pertained to actual books. As children, we were encouraged not to assume we'd hate a book before reading it and, by the same token, to understand that just because something looked "cool" didn't mean it was. The inside of the book—the true substantive character it has—is something we have to read through with an open mind before making any assumptions. I like to think this truth pertains to people.

Not everyone is born into the same situation, and no two people follow the same path from beginning to end. We must take time to get to know a person and look beyond the surface of their lives to really understand them. This comic recognizes that most people do not put this truth into practice. The main character's specific situation is not the primary focus of the comic, but rather it is the fact that everyone she knows only sees what they want to see and not who she truly is.

— by Joseph Castillo

Comics are such a powerful form of expression. Because comics are such a visual medium, the audience is required to really get involved with what is going on in the story. When Joseph and I realized that the story we created contained very universal themes, we relied heavily on the visuals of the comic and kept wording to a minimum in an effort to really bring out a closeness to the character.

The first week that Joseph and I arrived on campus, we knew no one but each other; the environment was different, the people were different, and we felt like outsiders. This feeling is what we attempt to present in this comic. Although we did not experience exactly what our protagonist encounters, we felt that her experience would be the most thoughtful way of expressing what people see and how they perceive and interpret others without actually knowing them. The comic revolves around the idea of who a person is versus how the world sees them. The final product, we hope, is something that the audience will be able to readily interpret and relate to.

- by Desi Alicea-Aponte



















Bird in a Cage is a 3D animated short film created by students in the arts and technology program at The University of Texas at Dallas. The short film tells the story of an inventor who creates a set of wings enabling him to fly, only to realize that he is trapped inside his laboratory.

I started work on the short in August 2011, and the final video was completed in summer 2013. *Bird in a Cage* is primarily the work of undergraduate students, although several participants graduated and continued work on the film during its twoyear production life. While the original creative team consisted of a mere handful of students, the final product is the cumulative work of more than 20 students.

- by Greg Slagel



To watch the film, scan this code or go to vimeo.com/74722109.

Director, preproduction, texturing, lighting and compositing, additional effects **Greg Slagel**

Additional animation Andrew Vasquez

Preproduction Ashley Davis

Rigging, additional animation Blake Sherill

Modeling, layout, animation, additional lighting **Cameron Kamenicky**

Concept art, storyboarding, texturing Cara Curley

Layout, additional animation Chris Elsea

Lighting and compositing, original score, sound design, mixing and mastering, voice acting **Chris Miller**

Layout David Budd

Texturing Griffin Marshall Huda Hashim

Preproduction, modeling Justin Wood

Additional compositing Kathleen Tran

Preproduction, preproduction sound Matt Prince

Animation, additional rigging Matt Thurman

Preproduction, modeling, texturing, additional rigging, additional effects, additional animation **Michael Bonnell**

Layout, rigging, additional animation Michael J. Sewell

Concept artist, storyboard artist, modeling, texturing Monica Deal

Preproduction, additional animation, layout Naveen Syed

Additional animation Santiago Andres Alarcon

Modeling, additional animation, production blog Sarah Simes

Additional texturing Sarah Wright

Effects Steven Vance

Additional modeling Vincent Lo

Patti Henry Pinch Scholarship for Undergraduate Research

The Patti Henry Pinch Scholarship is designed to assist undergraduates at UT Dallas with research and travel expenses by offering supplemental financial support of up to \$1,000 (half of the award will be provided by the student's school and half will be provided by Patti Henry Pinch Undergraduate Scholarship funds) on a competitive basis. Students should consult their research advisor in order to complete the application.

For more information, visit oue.utdallas.edu/research/patti-henry-pinch-scholarship.



Student Research Resources

UT Dallas offers a variety of research opportunities and resources. Engaging in research as an undergraduate has academic, personal and professional benefits. Research activities enhance analytical and critical thinking skills, which prepare undergraduates for the rigors of their profession or graduate studies. Learning how to practically apply academic concepts, discovering how to work independently, and experiencing how to function successfully as a team member are other benefits many students attribute to participating in undergraduate research activities. Exploring a discipline through research also allows students to make informed decisions about the career they wish to pursue. The Office of Undergraduate Education (OUE) and the Office of Research provide a variety of research event opportunities and other resources to assist both prospective and current undergraduate researchers.

For more information about undergraduate research opportunities, visit oue.utdallas.edu/research/student-research-resources.



