the Berkeley Carroll School

Proceedings of the Berkeley Carroll

4TH INDEPENDENT RESEARCH CONFERENCE

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Jane Moore
Director of Upper School

Robert Vitalo
Head of School

Welcome from the Editor

One of my favorite parts of science research is the lab meeting. It's a chance for the scientists in a lab to sit at a table and show each other what they've got—when you float your half-baked hypothesis or ask if your new data graph looks more like a straight line or a sine curve.

At a good lab meeting, thoughts are free flowing but not yet crystallized, and feedback is probing, thoughtful and constructive—people listen to each other and interrupt each other all at the same time.

This year, the Berkeley Carroll Science Research and Design Program has taken a step toward establishing our own tradition of the lab meeting. We've done so by making the 11th grade class (the middle year of this three year program) a full-graded course—a chance to meet six times a cycle with the same young scientists over the course of a year.

The authors of this journal are the first class to complete this 11th grade version of "Advanced Science Research and Design" and you can tell by their work how well they know each other's research and writing.

When you spend as much time together as these authors have, you can't help but know what your fellow researchers are up to; they've had countless chances to review one another's work, serve as subjects in each other's experiments and offer up suggestions.

And the echoes of all those lab meetings resound in the pages of this journal.

You can hear them in the discourse between Rasheed Evelyn and Sophie Hayssen on the topic of synesthesia, that peculiar mixing of the senses in which some people see numbers as particular colors. While Rasheed suggests you can't learn synesthesia, Sophie's results show that something not so different from it can develop over time.

Abigail Marin researched the general importance of stem cells, Julia Pike found a connection between stem cells and Alzheimer's disease and Alex Pachter found a connection between Alzheimer's disease and zebrafish.

Niamh Micklewhite didn't quite find evidence that artificial shark skin resists bacteria on the walls of Berkeley Carroll, but Sunny Birdi implies that colloidal silver just might.

Max Pisano shows us that if you listen closely enough and ask a big computer in just the right way, social media may tell you the price of a share of stock on the market. And if that's not enough, David Pachter's impeccably clear explanation of quantum teleportation suggests that it may be possible to transmit a signal through an unbreakable code.

The face-to-face lab meetings are over, but their work still resonates and I'm feeling really lucky that I can still hear it and share it with you.

Until next year,
Scott W. Rubin
Upper School Science Chair

Science Research and Design
SENIOR RESEARCHERS

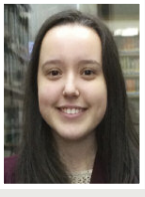
Sunny Birdi '15

Sunny's research is focused on bacteria that has adapted and developed resistance to antibiotics. The genes that allow resistance are passed on to future generations of microbes or can be transferred to other species. This raises ethical issues because scientists create medicines and doctors prescribe them in the hopes of eradicating these microbes that threaten our health; however, if the microbes develop a resistance, they can end up becoming more infectious. Sunny will present on this topic, and plans to conduct lab research about it as an intern at Rockefeller University's Summer Outreach Science program.

Rasheed Evelyn '15

Rasheed concluded his final year of research with a continued focus on grapheme color synesthesia, a neurological phenomenon where an individual's perception of numbers is associated with colors. He conducted a study that looked at potential theories for its cause, focusing specifically on whether non-synesthetes can acquire synesthesia over time. This research was particularly connected to the prenatal hypothesis. He plans to repeat his study with a larger sample size for an extended period so he can incorporate new research from around the world as it develops.

Sophie Hayssen '15



During her three years in the program, Sophie concentrated her studies on neuroscience and the brain. She specifically focused on synesthesia and its connection to cross-modal perception. For her final project, Sophie wrote a paper about her original research on how sensory perception changes over time. She would like to continue with this line of study in the future, incorporating multimedia artwork.

Abigail Marin '15

Abigail will present her research on stem cells and cancer. As part of her studies, she shadowed a post-doctorate fellow conducting research on Fanconi anemia in the Smogorzewska Lab at Rockefeller University. She has been accepted to the internship program at the National Institute of Health (NIH) this summer, where she hopes to gain experience researching stem cells and cancer.

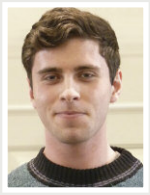
Niamh Micklewhite '15

For the past three years, Niamh has been researching sharks and their skin, which consists of scale-like structures called dermal denticles. She has looked specifically at synthetic shark skin as an antifouling solution to prevent biofouling, and more recently, at its potential use as an antimicrobial. This year, Niamh conducted a study where she examined the difference in bacterial growth between Sharklet, a smooth surface, and an everyday surface. At the Science Research and Design Conference, Niamh will present on her past and present research as well as the results from her study.

Alex Pachter '15

After thoroughly enjoying her neuropsychology Spring Intensive in sophomore year, Alex started exploring neurodegenerative diseases and topics like spinal neuron regrowth processes and Alzheimer's disease. In her junior year, she worked with Dr. Richard Kollmar in his lab at SUNY Downstate Medical Hospital studying the molecular genetics of otolith formation, which is associated with vertigo, in the zebrafish. She learned the process behind expressing proteins by using gel electrophoresis. Her final paper is the culmination of all of these studies: Alex explores the use of the zebrafish model in Alzheimer's disease. She will explain animal models in science and expose complementary proteins in the zebrafish that could help future studies on therapeutic drug developments for Alzheimer's disease.

David Pachter '15

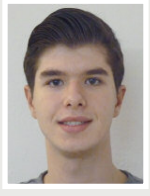


Over the past two years, David has been reading published scientific articles on the fundamentals of quantum mechanics, focusing specifically on the quantum phenomena of entanglement and teleportation. Quantum mechanics is a branch of physics that focuses on the nanoscopic realm of particles, and entanglement is, as Einstein called it, "spooky action at a distance. In his presentation at the Science Research and Design Conference, David will explain the quantum concepts he has learned, including how to teleport a particle.

Julia Pike '15

Julia's paper and presentation focus on cutting-edge research in the field of Alzheimer's disease, including its current treatments. Her studies included the broader topic of neurodegenerative diseases, as well as a month-long internship at the New York Stem Cell Foundation, where she aided the Alzheimer's team in creating RNA, staining cells and obtaining the results of experiments. In her sophomore year of the Science Research and Design Program, she explored many topics, including water purification technology, wave technology and different types of sustainable energy.

Maxwell Chase Pisano '15

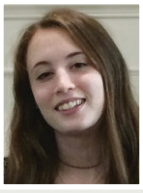


Maxwell was drawn to the topic of predictive analysis of the stock market using social media sentiment analysis through his interest in technology, finance and social sciences. In his research project and presentation, entitled "Predicting the Stock Market Using Sentiment Analysis of Social Media and Predictive Analytics of Structured and Unstructured Data," he explains how he predicts the stock market by using social media as a gauge and how analytics can predict trends before they happen. He also discusses the system's benefits and drawbacks. In the future he plans on continuing his research in the fields of predictive analytics and big data analytics to try to discover more uses for the ever-growing amount of data that users produce every day.

Samantha Schreiber '15

For her final project, Samantha will present on her experiences and findings from the Hurd Lab at Mount Sinai Hospital, where she helped researchers study the effects of heroin self-administration in rats. For the first two years of her Science Research and Design Program, she researched prosopagnosia—otherwise known as face blindness. This is a disease that causes people to not recognize the faces of others and sometimes even their own. Due to the lack of prosopagnosia research opportunities in New York, she wasn't able to conduct a final project about this, but she plans to continue researching the topic in college.

Lucy Shenk '15



Lucy's research focused on the psychology of procrastination. She initially studied sleep disorders and positive psychology and then switched to this broader topic, partially because she is affected by procrastination every day. She was relieved to discover that this is not a time management problem, but a real psychological issue that seriously affects roughly 20% of people in the United States. She conducted a pilot study with Berkeley Carroll students last spring, and a full study in the fall.

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Brain Plasticity: The Acquisition of Grapheme Color Synesthesia

by Rasheed Evelyn

Abstract

The purpose of the experiment was to test whether or not non-synesthetes could learn synesthesia or if exposure to graphemes could elicit synesthetic reactions. Non-synesthetes were tested using the synesthesia battery created by David Eagleman, a leading researcher in this field. The results of this experiment were measured based on the participants' responses to the battery. The completed questionnaire was reviewed along with subjects' overall scores for the battery. None of the participants' scores showed a significant improvement. From this one can conclude that it is not possible to acquire synesthesia. This conclusion supports the neo-natal hypothesis by indicating that synesthesia cannot be acquired after birth.

Introduction

Synesthesia is "a surreal blending of sensation, perception and emotion" (Ramachandran). Synesthesia is derived from cross-modal perception in which two different sensory modalities become intertwined (Weiss). In particular, grapheme color synesthesia (GCS) is a condition through which synesthetes (people who experience synesthesia) associate letters and numbers with particular colors. There are varying ways in which this condition may arise. Either a synesthete will see the color within their mind's eye, known as an association, or they will see the color directly imprinted on the grapheme (any letter or number), known as a projection. For each synesthete the pairings are unique in color, tone and hue. There are never two synesthetes with the same pairings (Eagleman). However, not all graphemes elicit pairings for synesthetes. Some synesthetes may only experience synesthesia for a small number of graphemes. Typically, the effects of GCS supersede font and size. If a particular grapheme is already depicted in a color, the initial color of the grapheme and the color that the synesthete pairs it with will blend. Both of these colors will affect the synesthete at the same time (Ramachandran).

Synesthesia does not have any noticeable drawbacks. For most, it is seen as an extra sense. In some cases, it may even be an advantage. People with auditory-visual synesthesia have a much easier time remembering music notes because sounds elicit colors. For grapheme color synesthetes there is a similar advantage. Remembering the spelling of particular words becomes especially easy if the synesthete can remember the progression of colors (Eagleman).

A condition like synesthesia is very reminiscent of something much more common: optical illusions. Both induce a change in perception by manipulating the senses.

Synesthesia is caused by an interaction between the fusiform gyrus in the pre-frontal cortex of the brain and the retina within the eye. The V4 region of the pre-frontal cortex helps the brain to process colors (Ramachandran). This region for synesthetes is believed to have an excess of neurological connections that was not efficiently pruned during development. These excess connections within the brain cause the involuntary interaction between the senses to take place. This is known as the neonatal hypothesis (Tomson). This is the leading hypothesis for how synesthesia is acquired. This is the prevailing theory, and as such, the potential causes for synesthesia have been limited.

The present experiment tested whether or not synesthesia could be acquired in non-synesthetes after birth. David Eagleman, a leading researcher in the GCS field, has created a synesthesia battery that tests for multiple forms of synesthesia. This battery has been an established tool for synesthetic research for quite some time. This battery is the gold standard within the field of cross modal processing and synesthetic research. This experiment is unique in that the battery has never been used for analyzing results of known non-synesthetes in the hopes of manipulating their responses to mimic those of true synesthetes. Using this battery, I want to understand whether or not non-synesthetes can learn grapheme color synesthesia. Would it be possible for a subject's results of this battery to improve over time? If in fact synesthesia is present through an excess of neurological pathways then this could not be trained in non-synesthetes and the participants could not achieve a score similar to synesthetes. Participants took the synesthesia battery on multiple occasions and their score was assessed.

This experiment was useful in furthering the discussion for how synesthesia is acquired. If synesthesia is acquired through the neonatal hypothesis the results of this test would be insignificant. A non-synesthete's progress with learning synesthesia would be measured by comparing the scores that they receive after the completion of the synesthesia battery over a given period of time. If the scores received in this battery improve, then it can be argued that a person's cross-modal abilities have been enhanced effectively, making them one step closer to being a synesthete.

The battery consists of three parts. The first part records participants' response to stimuli (graphemes). Subjects choose a color that they feel is best associated with each grapheme. There is a large variety of colors to choose from which accounts for the uniqueness of any given synesthete's pairings. All thirty-six graphemes are presented to the participants a total of three times randomly. The program records the consistency of response (Eagleman). The second portion of this battery tests for accuracy and reaction time. Graphemes are projected across the screen for exactly one second with a color already associated. Participants must choose whether or not this color matched the colors they chose in the previous section. Exactly half of the projected graphemes match ones chosen by the participant (Eagleman). For synesthetes, the responses that they make in this part are typically very quick because they know their synesthetic pairings so well. For others who attempt to fake synesthesia, the choices are much more difficult. The third and final part of this battery is a questionnaire. The questionnaire asks many questions about a participant's background. Some of the questions deal with family history or ask participants to rate how closely a given experience elicited synesthesia for them. The results of these three sections are combined into a large data sheet that helps determine whether or not a participant is a synesthete. Eagleman and his team have set standards of

expected values for synesthetes that are used as benchmarks in determining whether or not someone is a synesthete. This battery is usually taken on more than one occasion several months apart to ensure accuracy and consistency of results. The results for the various sessions are compared to create a final analysis (Eagleman).

The results of the grapheme color synesthesia battery will show large discrepancies between synesthetes and non-synesthetes. These discrepancies should be apparent in all forms of the testing results. Once the results of participants are acquired they are to be compared to the results from leading researchers' tests with synesthetes. Additionally the individual results for each participant will be compared to look for any significant change. In essence, has the participant come any closer to achieving the score of a true synesthete?

Materials and Methods

The synesthesia battery is posted online. Eagleman has made it accessible for all to determine whether or not they are a synesthete. Participants in the present study registered through email. Once an account was created participants chose the type of test that they would be participating in. In this case, they all participated in the grapheme color synesthesia battery for both colors and numbers. This study was done twice. Each of the times, the study is the same; however, the second set of results gives a basis of comparison. Participants are able to sign in and out of their accounts, resuming right where they left off. Between the first and second test-taking periods there is a one week gap.

Participants go through the steps of registering and inputting the appropriate information. There are two key elements within this step. One ensures that the researcher can view the results, and the other ensures that participants register for the appropriate tests. There is a long list of batteries each with a different function that allows for different tests. Participants register for the one associated with GCS that includes both letters and numbers. Secondly, once the test is selected, there is an opportunity to put in contact information for a researcher. Participants input the researcher's name and email into these slots. This ensures that the results received are viewable by the researchers upon completion. Results are automatically sent to researchers' accounts upon a subject's completion of the battery.

Results

The results of the synesthesia battery conducted by the Berkeley Carroll community does not show a significant difference to the results obtained by David Eagleman. In terms of the overall score, the in-house group scored an average of 2.02 that is comparable to the controls within the Eagleman group with the majority scoring between 1.5 and 3. To further support this conclusion, correlated t-Tests were completed based on the subject's scores on the color picker test for the battery. T-Tests produce p-values that indicate significance of results. This t-Test produced a p-value of .895 indicating that the results are insignificant. In order for results to be deemed significant the p-value would have to be less than .05.

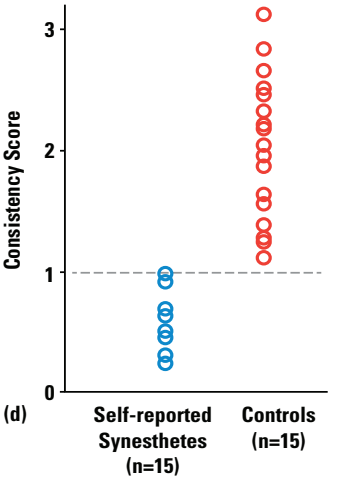

Figure 1

This shows the results of the GCS battery conducted by David Eagleman et. al. Taken from: "A Standardized Test battery for the Study of Synesthesia." This depicts the scores of synesthetes and non-synesthetes in his study. This set the baseline for determining whether or not a person is affected by synesthesia.

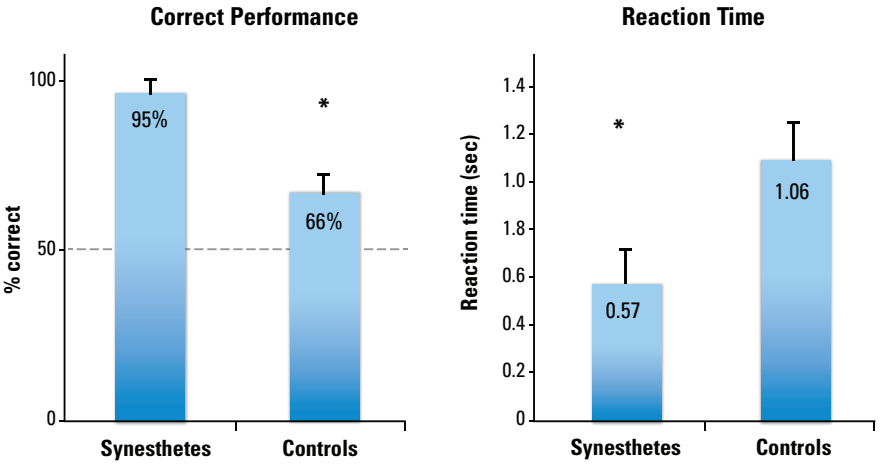

Figure 2

Figure 2 shows the results of self-reported synesthetes and a control group. The performance of these two groups is drastically different in the Speed-Congruency test. Taken from: "A Standardized Test battery for the Study of Synesthesia."

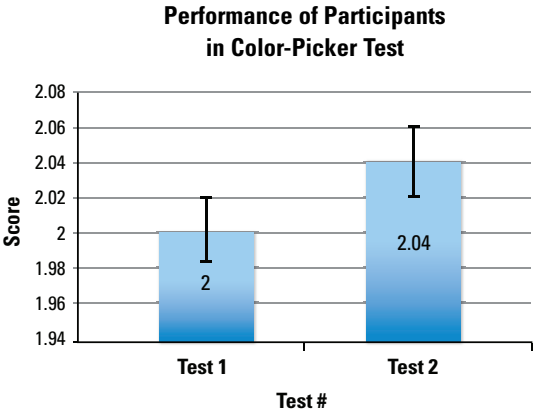

Figure 3

Figure 3 shows the participants' results in the current study for the Grapheme Color Synesthesia battery (average +/-SEM). Here is a comparison for the overall scores of the participants. In order for participants to be considered synesthetes their score must fall below one. This only happens for one participant.

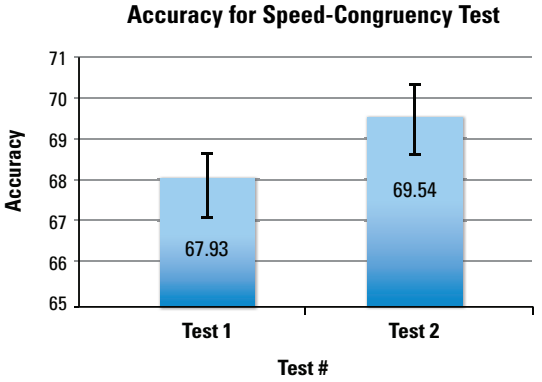

Figure 4

Figure 4 shows the accuracy of the participants' choices in the speed-congruency test (average +/-SEM). This is the typical percentile range found in non-synesthetes. Even without distinct grapheme-color pairings non-synesthetes achieve a relatively high amount of accuracy.

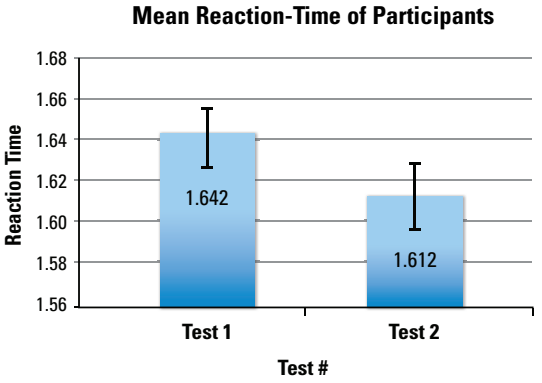

Figure 5

Figure 5 shows the reaction time of participants during the speed-congruency test (average +/-SEM). This reaction is much higher in non-synesthetes than in synesthetes.

Each of the participant's results on the Color-Picker tests shows inconsistencies. Between the first test and the second, the color choices are wildly varied for all of the graphemes. However, it is interesting to note that the color choices tended to be similar within one individual test. With one test, all the R's may have some variant of red for example. This does not stand for much though. The results of the tests need to be compared to each other. Looking at the individual results of a test do not help answer the research question because it does not give a base of comparability. The tests would only have been significant if there was a red hue for R throughout both tests.

The same results follow suit for the Speed-Congruency test. Eagleman's results forecast sixty-six percent accuracy while the in-house results show sixty-nine percent. In terms of reaction time the results of synesthetes are about half that of the non-synesthetic group. The in-house study mean reaction time was about 1.5 seconds. This was over twice the time taken by Eagleman's synesthete group.

The majority of participants' reaction times decreased between the first and second trial along with the average reaction time. However, no trends can be distinguished for the accuracy in the Speed-Congruency test. The accuracy from one test to the next increases yet this is not consistent for all participants. No clear trend can be found.

Conclusion

The results do not show a significant change in the scores on the synesthesia battery. There are no trends in the data that suggest that participants' scores were coming close to that of a regular synesthete's. From the results produced, there is not a significant enough shift in the scores of participants to suggest that synesthesia could be acquired. The results of the in-house study's participants are similar to that of a typical non-synesthetic group. Using this data, the neonatal hypothesis is supported. This data suggests that synesthesia is acquired during infancy when there are an excess of neurological connections within the brain.

Admittedly, the sample size in this experiment is less than ideal. In order to assert this claim more definitively a large sample size would have to be tested. In addition, having a large sample of tests for the participants to take would allow you to assess the trends more accurately for both the group and individual participants. There is one other discrepancy that lies in this procedure. Due to limited timing, the gap between tests was very short. Eagleman's report suggests at least two months in between testing. The longer gap allows the brain to recuperate. In effect, it gives the participants the ability to treat each test as their first time with no recollection of previous answers. Relative to Eagleman's suggestion, the tests are very close together.

Another research group has conducted a similar experiment (Bor). Their method includes using the same battery created by Eagleman. In fact, their study is so similar in that it even tests for the same type of synesthesia. However, the results of their experiment were in direct opposition to the conclusions found here. Their results did not prove that synesthesia could be acquired after birth however it did show a significant increase in the results. This allows one to postulate that over time, the brain could be trained so that synesthesia can be learned. Admittedly, there are some discrepancies between the sets of results. Granted however, this research is relatively new with that study published just weeks before the time of writing of this study. If the in-house study were to be conducted once more, a larger sample would have to be used in conjunction with a longer gap of time to allow for greater comparability.

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Predicting the Stock Market Using Sentiment Analysis of Social Media and Predictive Analysis of Structured and Unstructured Data

by Maxwell Chase Pisano

Abstract

My research project is "Predicting the Stock Market Using Sentiment Analysis of Social Media and Predictive Analysis of Structured and Unstructured Data." Over the course of three years, I investigated the fields of natural language processing (NPL)[1], machine learning[2], parallel computing[3], speech recognition and processing[4], sentiment analysis[5], and big data analytics[6]. The premise of the project was that social media provides us with ample amounts of data which have a numerical value that is yet to be assigned. Social media data is composed of unstructured data, usually in the form of text. This data in its raw form cannot be processed by computers to predict changes in structured data sets. So to overcome this obstacle, I utilized NPL and sentiment analysis to parse through data from five social media sites (Twitter, Facebook, Instagram, Stocktwits, Google) and two news sources (Yahoo News and Bloomberg.) By utilizing custom SQL databases, the data could be live streamed and stored. By then employing machine learning and NPL in combination of sentiment analysis through the use of a custom lexicon[7], I was able to obtain a numerical value for the weight that the words carried in the social media posts. These changes in score were then correlated with changes in stock prices. By utilizing machine learning, an algorithm was created and refined to predict the influx of the stock price using the sentiment of the social media post. Then, using data analytics and parallel computing, I was able to forecast data trends and predict, 30 minutes in advance, what was happening on the market with a high level of accuracy.

[1] The ability for computer to derive meaning from natural language input

[2] Algorithms that can learn and adapt from past data

[3] Helps in performing large computations by dividing the workload between more than one processor, all of which work through the computation at the same time.

[4] The ability for computer to derive meaning from natural language input

[5] Deriving the connotation of a word detecting if it is negative or if it is positive and assigning a numeric value to the words.

[6] Big Data is information that can't be processed or analyzed using traditional processes or tools

[7] A dictionary that has the words sorted by negative or positive connotation.

Introduction

Humans have always tried to predict the unpredicted. We strive to find out the unknowns and erase the odds. This is something that is part of our human nature. One system that man has tried to conquer and predict is the stock market. The stock market is one of the most vital parts of the free market economy. Everyday, billions of dollars trade hands from investors all over the world. Investors trade over 1.6 billion shares day, of which over 2,200 stocks that are listed on the various exchanges. What started as a small social club has transformed into a worldwide financial powerhouse. This market has mesmerized men and women of countless generations looking to get rich and frolic in the wealth that it provides. However, this market is quite unpredictable and reckless and can be compared to the proverbial "bull in a china shop."

But what if we were able to tap into the minds of the people trading on the market to better predict the market and beat the odds? This works if we follow this theory for market behavior: the consumer's confidence in that company and their feelings or sentiments will either raise or lower the stock price. In my opinion, this has to do with the fact that most people don't buy stock because of what the company makes or its financial reports but they buy it because of the name and the feeling that they associate with that company. For example, say that when you were a kid, you went fishing with your grandfather and every time you went fishing he gave you a Coca Cola. Now, when you are older you have this positive feeling about Coca Cola, and when you see the stock name, you associate those feelings with it, thus increasing the probability of you purchasing the stock.

This feeling is called sentiment. Sentiment is the connotation associated with a word, meaning the word either has a positive feeling or a negative feeling. Sentiment analysis of social media data allows unstructured data, which is text, to be quantified and predictive analytics to be performed so correlations between sentiment and stock prices can be found.

This necessity to predict the future has only been furthered by the wealth of information available to use for this prediction. Every day hundreds of thousands of gigabytes of data is uploaded onto social media. This surge in data that people upload to the Internet has to do with the new social norms where people feel the urge to share every moment of their waking day. While most of this data is pointless, such as someone posting a picture of his or her food or posting a "selfie" for the self-obsessed generation, you occasionally come across a diamond in the rough. These diamonds are found by using text-mining analytics.

This works by downloading the contents of the social media sites that you are looking at. In this case it is Twitter, Facebook, Stocktwits, and various news syndicates. This data is comes into the computer in plain text files, which are then fed through an analytics engine. The engine utilizes machine learning to read articles the same way humans do, which is accomplished with sentiment analysis.

Project and Research

Data is out there. The entirety of the Internet is comprised of computers communicating over network protocols pulling static data from servers. This data is returned in a format known as HTML (Hypertext Markup Language). HTML is a worldwide standard programming language for websites. HTML structures text and multimedia documents for viewing on websites and allows for other pages to link to each other serving as a directory service. HTML code is then interpreted by your web browser and presented to you with a GUI (Graphical User Interface). While this

may be useful for humans, computers prefer structured data for analytics and HTML is often unstructured, which means it is often full of errors. This has to do with the nature of the HTML structure and the advanced browsers that we have today.

The web browsers are able to compile websites with errors in the code, such as missing statements and tags, and still render the website correctly because HTML is able to calculate and plug in the missing data. The other issue with HTML is that HTML is basically a programming language that allows you to link many files together in a hierarchy and it lets the user see a graphic representation of it, instead of seeing it as a file in a directory on a web server. So for data analysis we need to call upon a different data protocol called API (Application Programming Interface), which is a fancy way of saying that instead of generating HTML files that people would understand we instead generate XML and JSON files. These files are similar to HTML, but differ because they are designed to be read by computers instead of people. XML and JSON files do not link to other files and the protocols are used to organize data into a structure[8].

These files are used on the server side of a website and the end user, when they are using a web browser, does not see these files. The files are used by websites to store data, which could be any type of data. This data is stored in this format because when encoded in this format it is both machine and human readable. XML and JSON are document-encoding standards that are used to store and transport data because they provide structure to the data enabling it to be easily stored in databases and transported for use in many different applications[9]. These files are not readably available, so websites utilize API to allow developers to access the data.

The way this works is fairly simple. Instead of the conventional way an HTTP (Hypertext Transfer Protocol) client works, where an end user makes a request over HTTP or to the web-servers at a server farm and these servers return HTML to the end user over HTTP, we are making a request from a web browser, and the request is made from one server to another server, hence the data is returned in XML or JSON format. The data is be requested by a server. That request is addressed to the server with the data on it. That server then returns the data back to the server that made the request in JSON or XML format. This data can then be entered into databases on the server and manipulated or visualized to then be returned to the end user in a way that they would understand, such as in charts or tables provided by the databases.

A database is a systematic collection of data and, since that data is organized data, management is less cumbersome. This management is performed by a DBMS (Database Management System). A DBMS is a collection of programs running on a server that enable the user to access a database, manipulate data in said database, and help in the representation of the data. For this project, a Relational DBMS was used to store the data. A Relational DBMS defines database relationships in a form of tables also known as relationships[10]. Relational DBMSes usually have predefined data types that they support and are useful for filtering out unnecessary data because the data allowed into the database is already predefined. What is unique about the relational model of databases is that the data is stored in table formats, which is an excellent format for data analytics. The Relational DBMS system that was used for this project was Microsoft SQL Server. Relational databases have a unique language for programming and that language is SQL, which Structured Query Language, pronounced as "S-Q-L"

[8] IBM SPSS Modeler 16 User Guide
[9] IBM SPSS Modeler 16 DB2 User Guide
[10] IBM SPSS Modeler 16 Social Media Analytics User Guide

or as "See-Quel" and is the standard language for Relational Databases. It can be used to insert, search, update, delete, and merge database records[11].

Databases were designed to solve a problem—you have data to store whether it is text or numbers or files. Data can already be stored on your computer as files say in word documents or spread sheets. Though just having data isn't the reason you need a database, and having data is not the problem though what comes next is the problem. The complications include: the size of data, the ease of updating the data, the accuracy of the data, the security of the data, the redundancy of the data, and the importance of the data. The first issue is the size of the data. What started off as just a few hundred lines in a spreadsheet program that worked speedily turns into a spreadsheet with millions of lines in it with actions taking forever to load. This happens because a computer does not use your hard drives to run and load a file. It uses the Random Access Memory (RAM) to run the program because the RAM is quicker than the hard drive, though the RAM is limited so as the quantity of RAM decreases file loading time increases[12]. So to counter that, you start to split up your data into smaller files. By doing that you run into a different issue and that is how are you supposed to find things quickly when everything is all over the place and not in one central location?

That brings us to the next issue, the ease of updating the data. Your data is now in many different files so what happens when you need to update an entry or change a field? You have to search through all the data to find what you are looking for and with a file-based system you are limited to only one person being able to edit the file at a time. Another problem is the accuracy of the data. Traditional spreadsheet programs or text files do not have any way of preventing users from entering incorrect data into the spreadsheet such as putting information in the wrong columns or incorrectly entering data or preventing fields from being left empty when entering data. This means that as time progresses you are left with data that is not accurate and data that can no longer be trusted.

Data security is a major issue in regard to data storage. When files are stored in a conventional way using text editing programs or spreadsheet programs you are left with an issue when you want to share the data with people. These systems don't allow you to share part of the data; you can only share all of it. Also, you are left with no way to track the changes and you are not able to prevent people from just viewing the data to being able to change the data meaning that the data can be incorrect or stolen. The next issue is the data redundancy such as having multiple entries for the same data. Conventional ways of saving data have no way of preventing the end user from duplicating data entries which can lead to the data having to be thrown out because now things are misrepresented.

The last issue is the importance of the data. In a situation where you are working on a file and all of a sudden the computer crashes and you lose everything. Now you are left with having to figure out what was saved and what wasn't. In mission critical applications where data basically makes up the entirety of a companies business, losing data can have severe consequences such as lawsuits or going out of business. Databases alleviate some of these issues. Databases allow us to store an "unlimited" amount of data where you are only limited by the size of the storage devices. It allows us to query that data at great speeds because of how the data is stored and how the system is able to anticipate data and understand the data that is entered. The data is accessible because multiple users can edit a database at a single time

and, because the database is stored on a server, any user with login credentials can connect to the server and access the data. The data is more accurate because databases prevent data from being entered into the incorrect fields by using rules and syntax that screen the data before it is entered to make sure it has the correct attributes and correct entities as the fields of the database tables. The data is secure because part of the DBMS system is an interface that allows for the creation of users with definable roles and restrictions for what the user is able to view or change. The data is absent of redundancy because of how the data is stored.

When data is entered, the text is turned into a Hash encryption key for identity purposes and if the Hash key of the data entered matches the Hash key of any data already inside the database, the database does not allow that data to be reentered. Lastly, because the data that is being stored is important and any issues entering it would spell major issues for a company, the database saves changes after any field is changed. Furthermore, databases are easy to backup and require little work to restore from a backup. This is all made possible because of the use of specialized programing languages such as SQL. It is because of these reasons that companies turn to databases to store information.

For this project the DBMS that was used was Microsoft SQL Server 2008 R2 Datacenter edition. Microsoft SQL server is a Relational DBMS, which uses SQL to program the data tables. Microsoft SQL Server allows task to be automated and allows access to the data from virtually anywhere with a data uplink. Microsoft SQL Server 2008 R2 Datacenter Edition was chosen as the DBMS system because it is reliable. It is one of the most used DBMS systems in the world. It runs on almost all versions of the Microsoft Server software and gives the ability to fetch data from outside sources, which is something that almost no other DBMS system can do. Also, what is unique to the Microsoft SQL Server 2008 R2 Datacenter edition is the use of Microsoft Visual Studio Programing interface, which takes a new approach at SQL programing. Instead of using PowerShell scripting in a Command Shell (CMD) window, which is basically just white letters on a black background. Visual studio on the other hand, allows programing to be looked at as building blocks. Coding is still done like it is done in PowerShell, but advanced features are now available such as the ability to run SQL scripts in a certain order by simply connecting the pieces of code in different configurations, as can be seen in figure 1.

In the experiment, data collection worked as follows. A SQL Server project was created and linked to a database. The project was composed of SQL script task. Each task had to be coded because there weren't, and still aren't, any plug and play solutions to achieve what the databases coded in the project did. That being said I will give a brief overview of how it worked, though the code and the logistics of the project will not be disclosed.

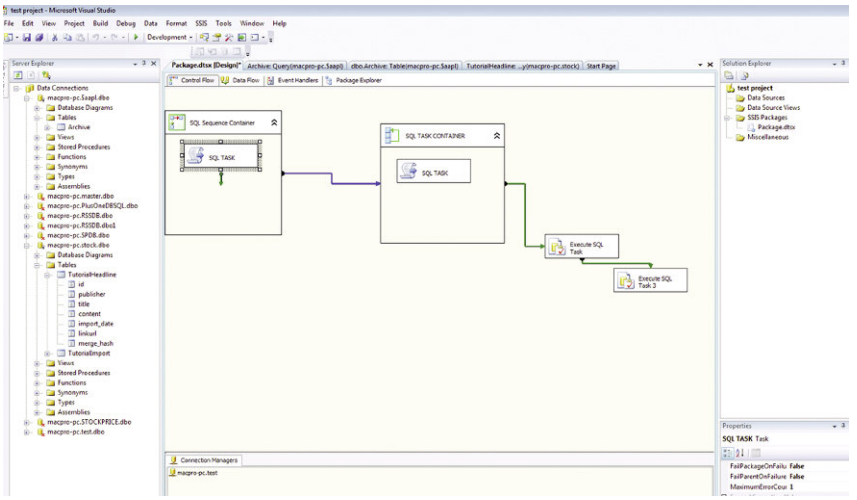
The first SQL task was used to make the API request for public post from my server to the websites of companies such as Bloomberg, Facebook, Twitter, Stocktwits, Google, Yahoo, and NASDAQ. This was done by utilizing SQL programing language to define the type of data that I was looking for, then to point the server to the right URL to obtain the data and provide the company servers that I was trying to obtain data from a trace back route, so that as data was being created on their end, if it matched my query, their servers would know how to send it back such as what format it needed to be in and the public IP address of my server. The next SQL task acted as the receiver of the data. When the request came back from the companies,

[11] IBM SPSS Modeler 16 Text Analytics User Guide

[12] IBM SPSS Modeler 16 Entity Analytics User Guide

Figure 1

Screen capture of Microsoft SQL Server 2008 R2 workflow



this task was the first task that touched the data. Think of this task as a person in a mailroom making sure that everything was going to the correct place. This task was also responsible for storing a temporary copy of the data that was sent by the company to the RAM of the computer so that the next task could sort through the data. The next SQL task opened up the JSON and XML files and prepared them for entry into the databases. It found out the way the data was structured and made sure that only data that matched the fields that were requested was in there stripping away all the junk and erroneous data. The next task stored all the data into a temporary database for further refinement.

Because of the nature of data there is a lot of junk and irrelevant information and because of the frequency of the request that was being made to the websites there was bound to be duplicates of data so the task also checked the final database to make sure that the data that was already in the database wasn't the same as what was in the temporary database. So the task processed all the data and deleted the unnecessary information and the duplicates. The following SQL task then transferred the data to the final database for it to be saved. The final SQL task saved the database and sent a notification to the next program to let it know that the task was complete and prepared the SQL server to run the task again every minute. Once the programming was complete, it was bundled up into what is called a package to be deployed on the server. Utilizing these packages I was able to make a template for the task, which meant that the only thing that needed to be changed in the code, when I wanted to add a new data source, was the first script task in the project to point it to the new URL of the data. This saved valuable time because it meant that I did not have to program the same thing over and over again on each server. This is another reason that Microsoft SQL Server 2008 R2 Datacenter edition was chosen as the SQL DBMS system for this project.

The amount of data that needed to be downloaded was a major concern for the project and based on the fact that data had to be requested, downloaded, sorted and saved in under a minute, it was decided that each source that I was going to download information would be

saved on different servers. The servers were virtualized, meaning that they ran on one server through a virtual environment, thus allowing five servers to run off of one server. The server was a Dell PowerEdge R920 4U rack servers with four Intel Xeon 4.87Ghz eight-core processors. The server had 64GB of DDR4 ram and quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support. To ensure quick data download, the server had four 10GB fiber optic ports connected to a switch, which connected to a modem from the ISP (Internet Service Provider). The server was on a rack in a climate-controlled room where the temperature doesn't go over 30 degrees. Each virtual server had Microsoft Server 2012 R3 Datacenter Edition with Microsoft SQL Server 2008 R2 Datacenter edition and Visual Studio 2014 installed. The Server had 24TB of storage space on six 4TB Western Digital Scorpion Black Series 7200rpm 3.5inch spinning hard drives. These drives were configured in Raid 0. Raid 0 is when the total numbers of drives are divided into two groups and the data on one group is mirrored onto the other group. This mirroring is done so that if there are any issues with the drives, the computer automatically switches over to the second set of drives. The server was also powered by APC units to supply backup power incase of a blackout.

This data was stored on the servers in an off-site location. None of the data processing was done on these computers. The data processing, sentiment analytics, and predictive analytics were all done on different machines. To alleviate the workflow, two Apple Mac Pro tower computers with 32GB of ram, quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support, two Intel Xeon 4.67Ghz eight core processors, four 1TB hard drives and 2 fiber optic ports were used. These two machines were running Microsoft Windows 7 Ultimate Edition 64 bit via boot camp. Each also had the Microsoft Office Suite installed and also Microsoft access server, Microsoft SQL Server 2008 r2, Microsoft Analytics server, Microsoft Data Viewer Server, Microsoft SharePoint Server, Microsoft Report Generator server, Microsoft SQL Server profiler, Microsoft IIC Server, IBM SPSS Modeler Premium 16.01, IBM DB2, IBM Data viewer, IBM SPSS Statistics engine, NVidia CUDA processing engine, IBM Congo's, Good sync backup, Forecast Pro, Microsoft Visual studio 14, R data package, python scripter, Eclipse java runtime environment, RSA cryptography program and TIBCO Data viewer. Each computer was connected via fiber optics to a custom built computer.

This computer was built to act as the master of the network and the three computers. This is called distributed computing where there is one computer that is the master and others that are slaves, which are called nodes. The master delegates task to the nodes. This allows data analysis task to be completed at a higher rate of speed. The nodes each process their part of the data and send their results back to the master, which then compiles all the data together for viewing. This form of computing is used in data intensive applications because multiple computers processing parts of data is faster than one computer trying to process all the data and then compiling it. The node network is connected to the master via fiber optic patch cables to enable speeds of data transfer up to 20GB per second. This required a fiber optic switch and fiber optic ports on the computers. All of these computers had this and a Dell fiber optic switch which was used because it could handle Category 6 cable and. it was also a smart switch, which meant that the data paths and the connections between computers could be managed to ensure the most efficient data speed was achieved to prevent network latency.

To save space, a Mini ATX chassis and motherboard were used for the computer. Mini ATX is a form factor of motherboards and is used for computers that do not need to take up a lot of space. The parts used to build this computer were an Asus Mini ATX rampage mother-

board, quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support, an Intel Core i7 3.0 GHz 8 core processor, 32GB of DIMM DDR4 RAM, one Samsung 840 series 1TB 2.5 inch solid state hard drive, one OCZ vortex 1TB 2.5 inch solid state hard drive, one 1TB western digital scorpion black series 2.5 inch 7200rpm spinning hard drives, a Corsair 1200 Watt ATX Power Supply Unit, and a Cool Master elite Mini ATX case. This computer was running Microsoft Windows 8.1 Pro Edition 64 bit. The software on the computer was: Microsoft Office Suite, Microsoft access server, Microsoft SQL Server 2008 r2, Microsoft Analytics server, Microsoft Data Viewer Server, Microsoft SharePoint Server, Microsoft Report Generator server, Microsoft SQL Server profiler, Microsoft IIC Server, IBM SPSS Modeler Premium 16.01, IBM DB2, IBM Data viewer, IBM SPSS Statistics engine, NVidia CUDA processing engine, IBM Congo's, Good Sync backup, Forecast Pro, Microsoft Visual studio 14, R data package, Python scripter, Eclipse Java runtime environment, RSA cryptography program and TIBCO Data viewer. The only difference between the versions on this computer and the other computers was that the version on this computer was for the master while the other computer had the version for the nodes.

After the data was downloaded and sorted on the servers, the computer node network connected via a NAT VPN connection. This allows the computing nodes to access the databases on the servers. The is done through the NAT VPN connection, which makes the computers think that the remote and local servers are on the same Local Area Network (LAN) network. This can only be achieved if you have a static public IP address. Once the computers connect to the servers, Microsoft SQL server is launched on the computers The Master computer delegates a certain database to each of the computers. Microsoft SQL server is used on these machines not only to act a redundancy in case there is an issue downloading the data or if the internet goes down, but when processing data, it is more efficient to save the data to the computer before processing, because that way the latency of the network connection does not need to be a factor that has to be accounted for in the data modeling.

After the data is stored into the SQL server I then used IBM SPSS Modeler Premium to start analyzing the data for sentiment. IBM SPSS Modeler is a compiler that allows you to generate scripts for the Statistical programming language R. What separates IBM SPSS Modeler from a lot of other software suites is that with IBM SPSS Modeler you are able to utilize the GPU for the processing of the data. So once the data is loaded into the SQL server by using SQL scripting the data is exported to IBM SPSS modeler. That task is the first node of script task. After the data is brought into SPSS, the next node strips away the unnecessary information from the data such as URLs, hash keys, and user credentials. This is also done by using a SQL script to strip data from the databases that were imported into the system. The next node utilized a C# script to initiate the sentiment analysis.

Sentiment analysis is a new study of data that has sprung up with the surge of consumer data that is available. Sentiment analysis is the process of using computers to detect the polarity of text. The polarity of the text determines if the text is negative positive or neutral. This is also known as opinion mining where text is used to derive an opinion from text to draw statistical conclusions. So say you wanted to find out how people on Twitter felt about the stock Apple? We would use sentiment analysis to determine if their mood is positive, negative or neutral. Say we have the Twitter post "$AAPL: Stock sunk 2.8% biggest slump since Oct how most valuable tech company fall 2.8% #Losing Streak #SELL%#BEAR"? If we analysis it closely and break it up to smaller chunks, we start to see some data. First the computer scans each and

every word individually with no context of words that are following or before each and every word so each word becomes its own entity. After it is done processing, a color-coded replica of the tweet is returned as we can see below.

$AAPL: Stock sunk 2.8% biggest slump since Oct how most valuable
tech company fall 2.8% #Losing Streak #SELL%#BEAR

After looking at the output, it is clear that "sunk," "slump," "fall," "losing streak," "sell" and "bear" are all negative terms represented in red. "Biggest" and "valuable" are positive terms represented in green. The rest of the terms are neutral, represented in black. Words that are next to each other and have opposite meanings are underlined since this can change the meaning of one word.

$AAPL: Stock sunk 2.8% biggest slump since Oct how most valuable
tech company fall 2.8% #Losing Streak #SELL%#BEAR

When compared to the first run-through without context, we see that the computer now added one term to the positive list and realized that biggest is no longer positive because it is a modifier for a negative phrase, so it is emphasizing the phrase. Next the computer assigns a value to the words based on positive negative or neutral. This is based on a rule that is pre-set and determines when it is appropriate to apply a certain value to the word. The rule was as followed:

Red = Negative sentiment words

- %#BEAR= This will be worth -2
- (Insert any word in red)= is worth -1
- #(Insert words after hash tag)= -.5

Green = Positive Sentiment Words

- %#Bull= This will be worth +2
- (Insert any word in green)= is worth +1
- #(Insert words after hash tag)= +. 5

Side note

Words underlined:
one is negative one is positive. When read in context, the phrase will have an overall positive or negative score be worth double points

Next the computer scans the sentence again, this time counting the number of positive words, negative words, neutral words and gives us a total word count. Then it scans the hash tags and tells us whether the hash tags are positive or negative. The results are then spit out into a table as reproduced below.

Positive Words	Negative Words	Neutral Words	Total Words	Positive #	Negative #	Total #
3	7	6	16	0	2	3

Points Negative	Points Positive	Overall Raw Score
-9.5	3	-6.5

The computer knows the whether a word is positive or negative based on the lexicons that are loaded into the computer. The lexicons are created by humans who classify words into three categories: positive, negative, and neutral. These lexicons also list what part of speech the word belongs to such as if it's a verb, noun, modifier or adjective. The list to includes the part of speech because it's important for the second run-through of the system.

On the second run-through, the computer pulls up the advanced version of the lexicon with the parts of speech and that is when it starts to find the connection between words and calculates how words affect other words in the tweet. The next run-through calculates the sentiment score. After that, the score is calculated on a rolling schedule, which means that the results are cumulated so they represent the trends of the past 7 days. This allows for a more accurate model to be produced by the forecasting software. After a sentiment score is obtained, it is graphed on a scatter plot with minute updates. This allows for the data trend to be visualized and thus then allows for the forecasting of the data. This process outputs two graphs one of predicted sentiment values and one of current sentiment values.

Next stock data was loaded into IBM SPSS statistics engine and a real time graph was produced using the data points. This graph was a line graph. Then predictive data points were generated as the price was forecasted. The final step was to use an algorithm and regression modeling to predict how the sentiment caused the price stock price to fluctuate. Finally after the data was modeled, a price was predicted based on this regression model that uses sentiment as a variable that affects price.

Materials

To conduct the research project many materials were needed. Because of the scale of the data that we were processing, the cost of the hardware and software and the amount of analytics required to understand it and come to a conclusion, your average consumer is unlikely to be able to replicate this experiment.

The ultimate goal of the project was to predict the stock price of any given stock on the market. In my case it was Apple, Inc. Because of the popularity of both the stock and the brand, there was a lot of data to process—almost 300,000 tweets per day. It was decided it would be best thing to use distributed computing to complete the task. That way we could spread the load of the task to the different machines.

For this project the material list is extensive and expensive so the probability of this project being replicated is slim. While the price will not be disclosed, the material list is here and if you so desire you could calculate the cost. (Software prices change every year and there are always fluctuations in pricing.) The materials are as followed:

- One Dell PowerEdge R920 4U rack servers with four Intel Xeon 4.87Ghz eight-core processors, 64GB of DDR4 RAM, quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support, four 10GB fiber optic port PCIE card, and six 4TB Western Digital Scorpion Black Series 7200rpm 3.5inch spinning hard drives.
- Two Mac Pro tower computers, 32GB of RAM, quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support, two Intel Xeon 4.67Ghz eight core processors, four 1TB Western Digital Scorpion Black Series 7200rpm 3.5inch spinning hard drives and 2 10GB fiber optic port PCIE card

- One custom built computer with Asus Mini ATX rampage motherboard, quad SLI NVidia Titan Black Series 12GB graphics processers with HPC support, Intel Core i7 3.0 GHz 8 core processor, 32GB of DIMM DDR4 RAM, one Samsung 840 series 1TB 2.5 inch solid state hard drive, one OCZ vortex 1TB 2.5 inch solid state hard drive, 1TB Western Digital scorpion black series 2.5 inch 7200rpm spinning hard drives, Corsair 1200 Watt ATX Power Supply Unit, and a Cool Master elite Mini ATX case.
- Three Dell Network Switches
- Three UPC battery backup units
- Microsoft Server 2012 data center edition- 1 licenses
- Microsoft SQL Server 2008 R2 Data Center edition - 4 licenses
  - Microsoft access server- 4 licenses
  - Microsoft Analytics server- 4 licenses
  - Microsoft Data Viewer Server- 4 licenses
  - Microsoft SharePoint Server - 4 licenses
  - Microsoft Report Generator server - 4 licenses
  - Microsoft SQL Server profiler- 4 licenses
  - Microsoft IIC Server- 4 licenses
- Microsoft Windows 7 Ultimate Edition 64 bit- 2 licenses
- Microsoft Windows 8 Pro 64 bit- 1 license
- Microsoft Office Suite 2013- 4 licenses
- IBM SPSS Modeler Premium 16.01- 1 copy
- IBM DB2- 1 copy
- IBM Data viewer- 1 copy
- IBM SPSS Statistics engine- 1 copy
- NVidia CUDA processing engine- 1 copy
- IBM Congo's- 1 copy
- Good sync backup- 1 copy
- Forecast Pro- 1 copy
- Microsoft Visual studio 14- 4 licenses
- Microsoft Visual Studio 12- 4 licenses
- Microsoft Visual Studio 10- 4 licenses
- R data package- 1 copy
- Python scripter- 1 copy
- Eclipse java runtime environment- 1 copy
- RSA cryptography program- 1 copy
- TIBCO Data viewer- 4 licenses

Data

The amount of data that had to be collected was tremendous. The data was being collected every minute of the day, seven days a week. So in order to understand this data, it had to be visualized. This visualization on the data that was collected was presented in the forms of graphs and tables. Because of the size of the data; every hour 60 data points were created, which means that 1440 data points were created every day; 10,080 data points were created

every week; 44,640 data points were created every month. The experiment lasted for five months which means that 223,200 data points were created over the course of this experiment.

As you can imagine trying to graph all that data on one page would firstly make seeing any slight changes almost impossible and secondly the graph would have to be shrunken to a size that would require a microscope to read. Since that would not do any good for anyone, the data was presented in snapshots., meaning that the data from one hour before was graphed with the new data. This allowed for the data to be understood and for the miniscule changes in price and sentiment to be seen. Also these snapshots were handy for checking the integrity of the algorithm. To put everything into perspective (Scott –Is this what he means?) these one-hour snapshots were only 0.00026882% of all the data that was available to be graphed. Along with other tools such as real time visualization tools and data tables, the data could be viewed while it was processed by the computer. The following are screenshots and graphs that show the social media data and the stock market data and how the data was graphed and how it was predicted.

Figure 2

Social Media Insights graphs: Using R and Python a dynamic graphing platform was created thus acting as a dashboard allowing for real time visualization to take place. These graphs show the number of tweets over the course of the day, the amount of messages every hour, the top hash tags related to the stock that was searched for, the top social media users who are using that hash tag. It also shows the devices that the users are using to post their comments to social media while also showing how much of the content is original or shared content.

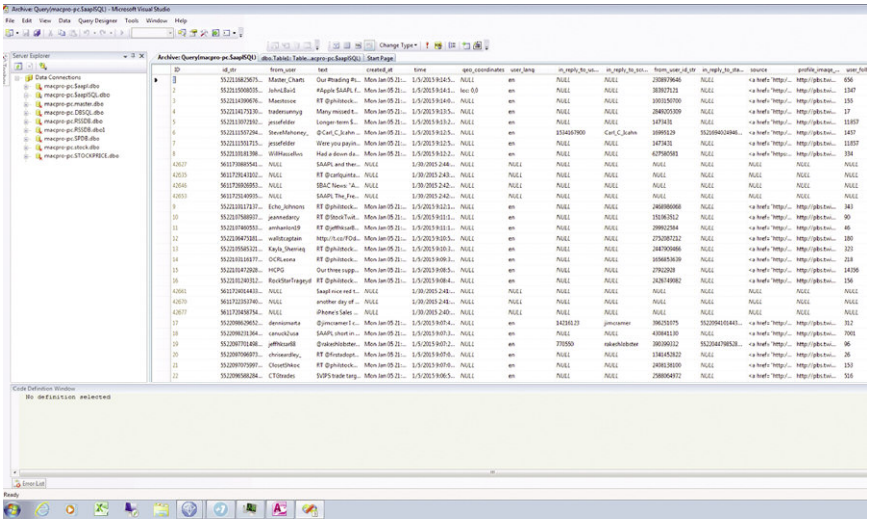


Figure 3

Microsoft SQL Server 2008 R2 Data Center Data viewer: This is screenshot of the SQL Server data viewer. Using this data viewer the data that is being collected by the SQL scripts can be viewed. In this screenshot is the viewer for all the social media data. The data is presented in tables though all of the fields are locked so no editing of the fields can be done. This is to ensure that the data is accurate because the data cannot be tampered with or changed after it is imported into the system.

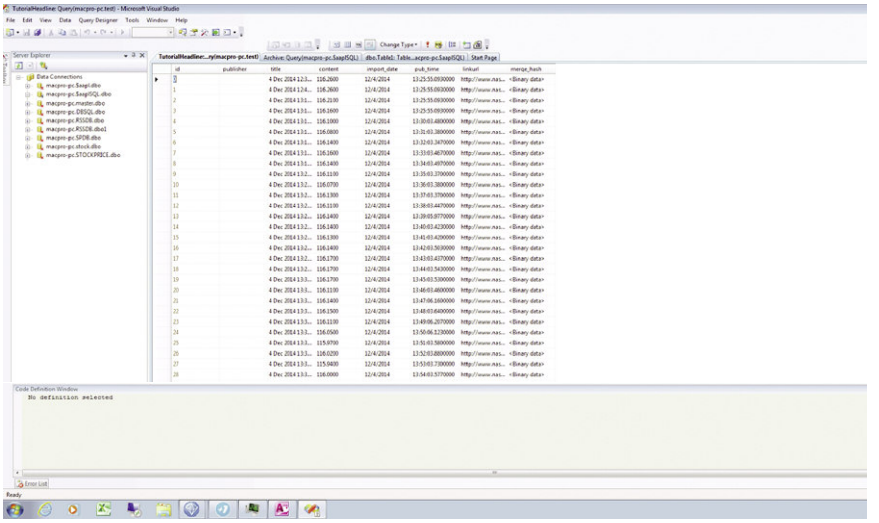


Figure 4

Microsoft SQL Server 2008 R2 Data Center Data viewer: This is screenshot of the SQL Server data viewer. Using this data viewer the data that is being collected by the SQL scripts can be viewed. In this screenshot is the viewer for all the stock market data. The data is presented in tables though all of the fields are locked so no editing of the fields can be done. This is to ensure that the data is accurate because the data cannot be tampered with or changed after it is imported into the system.

Conclusion

The results that have been collected so far have been substantial. It was shown that when there was an increase of mentions of a particular company, in this case Apple, there was around a 7% increase in the trading volume of Apple stock and about a 0.75% to 1% increase in stock price. The predictive models showed this rise anywhere between 5 to 15 minutes before the actual price increase in the market, thus giving investors enough time to act on the changes in the market. While the accuracy of the price hovered at around 60% to 75%, it was always plus or minus $0.05 to $0.10 of the actual trading price. Though the predictions of trading volume was accurate about 70% to 80% of the time and was within plus or minus 100 to 500 shares. With that being said, I would have to say that the overall outcome of the research was a success because while price and trading volume was semi accurate I was constantly able to predict if the price was going to go up, down or hold based on the sentiment roughly 90% to 95% of the time thus giving insight and order to a rather unpredictable market.

Data was collected over the course of five months. Stock prices were collected every minute from 9 am to 4 pm everyday. The social media data from Twitter and Stocktwits was collected every minute of the day, and every day of the week. News headlines were also collected on the same schedule. These news headlines were collected from Reuters, Bloomberg, Yahoo Finance, Wall Street Journal, and CNBC. Using this data, I was trying to find a correlation between the stock price and the sentiment of the social media post, which in turn was used to predict a change in price. In order to do this the predictions needed to lead the market, meaning they had to show a price increase before it happened on the market, although the predictions

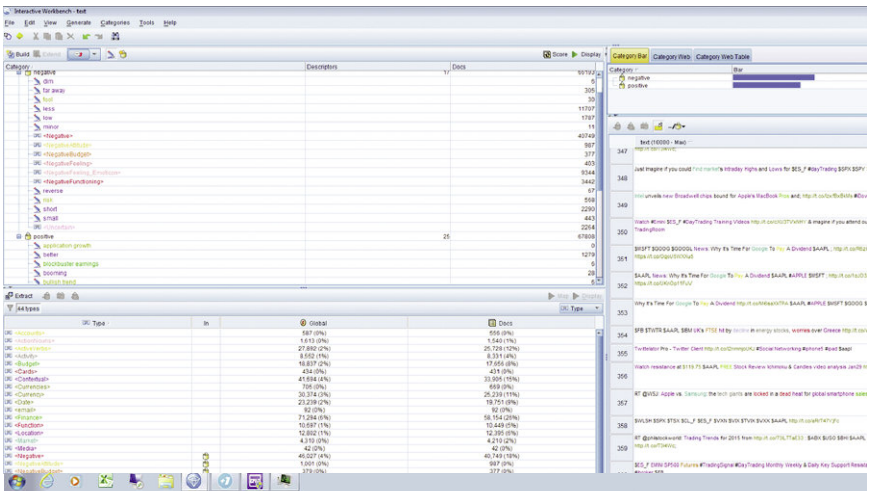


Figure 5

IBM SPSS Modeler 16 Text Analytics Node Viewer: In this screenshot is the Text analytics node viewer. This viewer allows for the social media data to be viewed. It also allows for investigating the effectiveness of the lexicon and the algorithm. What this Viewer shows is the sentiment score presented as a bar graph. It shows the social media post with different parts of the sentence highlighted to represent the different parts of speech and positive and negative sentiment. It also shows all the concepts that were extracted from the tweets and shows what concepts and make up positive and negative sentiment.

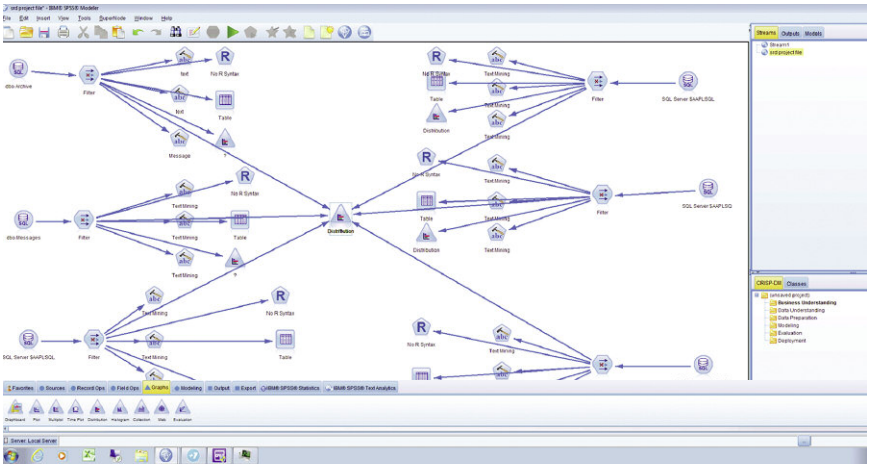


Figure 6

IBM SPSS Modeler 16 Visualizer: This is the interface that visualizes all of the connection of all the pieces of code for modeling. It breaks the pieces of code up into nodes and allows for code to be repurposed. This visualizer shows the connections between the different pieces of code that goes into gathering the sentiment.

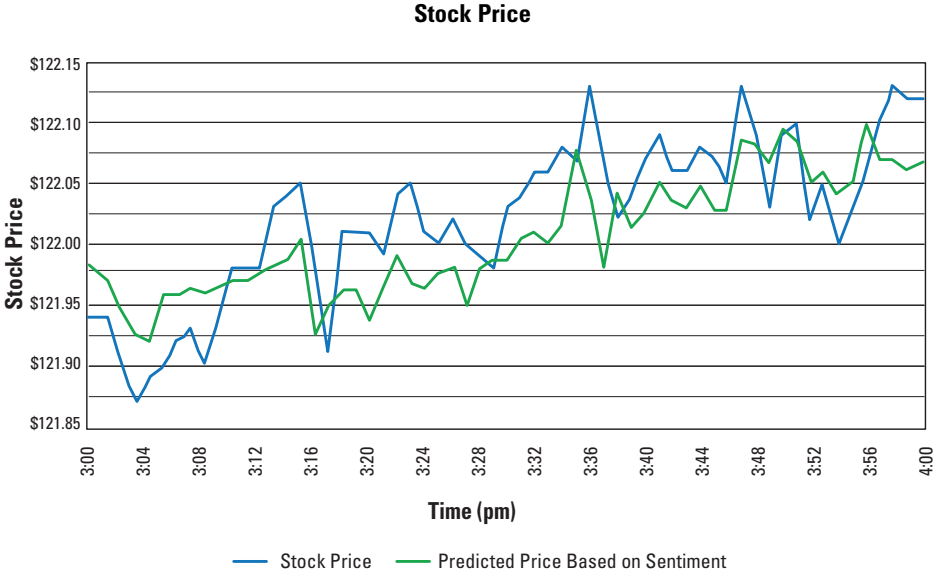


Figure 7

One-hour snapshot of Stock Price of AAPL vs. predicted price based off of sentiment score. This graph graphs the price of the AAPL stock on February 10th from 3pm to 4pm. The graph shows the real-time price of the AAPL stock in blue and shows the price that was predicted based off of the sentiment. This price was derived by using an algorithm and as it can be seen the predicted price was similar to the actual price in terms of the patterns and that the price was off by about plus or minus $0.05 to $0.10 cents.

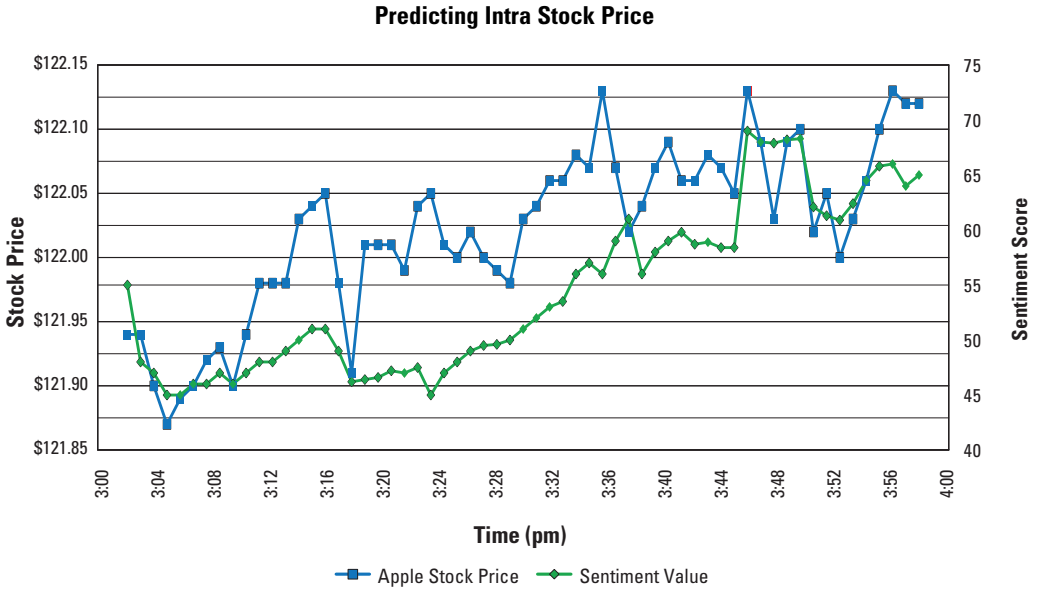


Figure 8

One-hour snapshot of Stock Price of AAPL vs. Sentiment Score. This is a graph of the price of AAPL, which is the stock symbol for Apple against time. On the secondary Y-axis the sentiment score in percent's is graphed against time. The sentiment score was classified, as 50% is neutral sentiment meaning that the sentiment is neither positive nor negative. Anything that was below 50% is negative sentiment meaning that the overall sentiment for the stock was negative and anything that was above 50% meant that the overall sentiment was positive. This was a collection of 60 data points from February 10th from 3pm-4pm.

are only as good as the data that was collected. This draws us to the major issue with Twitter and with the name "Apple". Firstly, because of the popularity of Twitter there are over 288 million users who post over 500 million tweets per day. There is a lot of junk on it and not every tweet has relevance nor is every tweet going to the needle in the haystack that will give us the insight that we need to predict the price of the stock. Also because of the way that the Twitter platform is set up with the ability for people to re-tweet a post that they feel is significant, there is a lot of redundant data, meaning that the same tweet could be counted multiple times in the sentiment calculation.

This problem is a double edged sword. While it may cause the sentiment scoring to be skewed due the immense number of times a tweet has been reposted, it can also show us that the tweet has some significant and some weight because if the tweet is being retweeted multiple times, the information contained in it most likely holds significant information. Though that brings up another issue: 5 percent of Twitter users or about 20 million users are bots . People can use bots to get retweets or to make it look like their profile is more popular. So if a user does not actually like the information but is using a bot the information may appear more popular than he actually is. Though if tweet gets enough re tweets it could become trending and than the false information could be perceived as real which is something that happens often with Apple usually regarding product speculations and new releases.

[13]Twitter, Corporation

[14]Business Insider, Inc.

Another issue is that "Apple" is a pretty common term so that meant that most of the tweets had nothing to do about Apple, the company. These tweets, which were garbage for predicting the stock price of Apple the company, included everything from "My Grandmother makes the best apple pie" to the "Damn look at her apple bottom jeans". Though this is where entity analytics come into the equation with entity analytics. Entity analytics allows the tweets that are garbage to be weeded out and not counted in to the sentiment analysis. Entity analysis works by seeing the association between the words in the sentence. It can tell the difference between words and how the words are used such as if they're nouns, pronouns or verbs. That way it can tell if it is mentioning Apple the company or apple in any other context. This prevented irrelevant data from being collected. However, this could lead to some issues with the overall quality of the predictions. Because of so many different variables and sentence structures, the system isn't 100% perfect. It could miss a few tweets, although in the whole scheme of things, a few tweets that are irrelevant doesn't affect the prediction by more than .03% , which is so miniscule that it isn't something to worry about.

When you then compare the results of an API request of the tweets of a pharmaceutical company, for example, or a company that serves a very small niche and doesn't have a lot of attention, all of the noise that you see with Apple is eliminated, but you also lose a lot of data. With Apple you could have 300,000 tweets a day, of which 220,00 had relevance. With some of these more obscure brands, there were about 1,000-2,000 tweets a week about them and only about 50 to 100 tweets that were absolute junk. This also has to do with the obscurity of the names that some of these companies have because names like Pfizer, GlaxoSmithKline, and AstraZeneca don't usually come up in a conversation. This means that the person that wrote a tweet with one of these companies' names in it, didn't do it by accident; they did it because they had something to say about the company and it was something of relevance. Though with refining of the entity analytics I feel that the noise that was undetected on the Apple stock could be detected thus making the prediction models even more accurate.

After completing my research and sifting through the data I have came to the conclusion that there are a few challenges that come with trying to predict the stock market with tweets. The first issue is that the information that was pulled from Twitter is information that is available to everyone. This means that there is no technical advantage to that piece of information now because it is available to everyone. Therefore, you have to assume that everyone knows about it and if everyone knows about it, key information like the current time and price that the stock is trading at already takes into account the news that is out there even though it is how you perceive the information that really affects the price. For example where one person might look at a blue circle and say it is a blue circle, another person may say it is 140 blue triangles. While both answers to the question may be correct, the way the person perceives the information is different.

The second downside to using Twitter is the credibility of the information on Twitter. Just because it is on Twitter or the internet doesn't mean it is true. Just because some random guy in the middle of nowhere said that Tim Cook is stepping down as CEO of Apple or some other guy claims to have some "insider" information regarding the release of a new product or a devastating event happening at a company doesn't make it true. Which is why when we want credible information we usually turn to the news. Though relying on the news means that you have to accept that there is a latency in the information because in order for the news to report

it, they have to make sure the story is true which in something as fast moving as a stock market by the time you find out if its true or not, the information is irrelevant and has already affected the price of the stock.

However that leads to the other side of the argument: if something is posted on social media and enough people follow it, repost it, and share it with friends, even though it is fake it could have the same effect as a true news story. This is because people might assume that it is true credible information since so many people are sharing it. That fake information though can still affect the stock price and could potentially cause greater effects on the price than credible information because things could easily be blown out of proportion. Also say you do come across credible insider information. Because mostly everyone posts on social media websites that are public, that insider information is no longer really insider information. This is why it's a horrible idea for one to share insider information on social media in the first place. It isn't private and because of the massive amounts of junk on the social media websites it only makes it harder to try and find that data.

Another downside is that because of social media website users are vulnerable and everyone wants to try and find information that could help them gain a competitive edge, social media sites can be used maliciously by people who want to drive the price of a stock down so that they can then buy it low and sell it for a higher price. This has to do with the herd mentality that people on social media sites have. They are followers and don't often think for themselves so they share and believe what other people say thus allowing the fake information to be spread no matter how ridiculous it is.

The last downside is the price of the system to achieve the sentiment analysis and the predictive analysis. For the average Joe Investor who is sitting on one or two shares of a company it doesn't make sense for them to invest in a system to predict the market. This is because the potential gains that they will get using the system will be far less than the cost of the system. They would be better off investing the money that they spent on the system because they would have a better shot of growing their money. But that is not the case when it comes to high speed trading firms and huge hedge funds that have tens of thousands of shares. The price changes in the stock that were seen over the course of a day were on average $0.10 to $0.15 cents, so when you multiply that by a few thousand shares, the system pays off.

Though there are some upsides. Firstly you are able to get an idea if the stock's price is going up or down before the price. This had to do with the sentiment and also the mass amount of social media posts because as sentiment rose and the chatter and mentions of the stock rose, the price rose slightly. This can also be seen when you are watching CNBC; the second the anchors start talking about the stock, you see slight movement in it, usually positive movement. While there hasn't been any research to support it, it is skepticism and intuition that has led me to this conclusion.

While the market will always be unpredictable, using natural language processing (NPL), machine learning, parallel computing, speech recognition and processing, sentiment analysis, big data analytics, regression modeling, and entity analytics, a consensus of the market sentiment is something that can bring some clarity to the market and lead to a slight advantage that in the long run can really pay off. Clearly this is just the beginning of what is possible with this technology. As social media grows and computing evolves, only time will tell what will be possible in the future.

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Literature Review of Quantum Entanglement and Teleportation

by David Pachter

Abstract

This paper will be concerned with teaching fundamental concepts of quantum mechanics, quantum entanglement, and quantum teleportation. Although in Young's double-slit experiments, particles such as electrons and photons individually make particle-like impacts on viewing screens, before impact they travel as waves. They are not tiny spheres but instead are spatially extended field quanta, or bundles of energy, that don't have a definite location until impact. Before impact, "particles" travel as a spread-out field before they encounter numerous atoms simultaneously. Upon impact, the field collapses to atomic dimensions, causing the particle-like impacts. However, they can only interact with one atom at a time, and since the single atom the particle interacts with is random, uncertainty and randomness is inherent in quantum mechanics. Because of this uncertainty, no definite outcomes can be foreseen even when all initial conditions are known, and thus in quantum mechanics we can only speak in probabilities—we can only give the *probability* that a certain event will occur rather than predict with certainty, for example, the time in which a baseball will hit the floor. The probability of different events occurring is embodied in the wave of a particle, and thus this wave can be said to be a probability wave. A probability wave is a combination of all the different possible locations of a particle, in other words a "superposition" of all its possible locations, wherein the probability wave is larger over areas where it is more likely to interact. A more accurate way to visualize a single particle, or field quanta, is as a one-particle wave packet because it shows the wave and probabilistic nature of particles as they exist before interaction. This wave packet description of particles can be used to describe "entanglement". Entanglement is when two particles exist in a single-wave packet, where the interaction of one particle instantaneously collapses the entire wave packet no matter the distance between the two particles, instantaneously affecting the second particle in a manner perfectly correlated to the first particle. Because entangled particles have this correlated relationship, they can be used for quantum teleportation. In teleportation, the properties of a third particle—other than the two entangled ones—are teleported. In essence, the third particle's properties, which are to be teleported, are destroyed and then recreated in one of the entangled particles. This has some real-world applications such as cryptography and quantum computing.

Introduction

Quantum mechanics is defined as the physics of the nanoscopic realm[1], such as sub atomic particles. Within this large field, my specific study is focused on the phenomenon of quantum entanglement and one application of this phenomenon: quantum teleportation. To understand these concepts, we must first understand certain principles of quantum mechanics, and how in

many ways, the world of particles behaves differently from the macroscopic world humans experience. These concepts include field quanta, uncertainty, and superposition. To understand these concepts, they will first be introduced by examining a classic experiment and its variants: Young's double-slit interference experiment using a light beam, an electron beam, and then repeating these experiments with time-lapse photography.

In the double slit experiment (Figure 1), Young shone a light through two parallel slits and observed the interference pattern it made on the viewing screen (Figure 2). What does this interference pattern tell us about the nature of light? An interference pattern is what results when two or more waves collide, also known as interfering. When the peaks (crests) of two waves interfere, the peak of the combined wave is the summation of the two peaks of the individual waves[7] (Figure 3a). Similarly, the troughs of the combined wave is the summation of the original waves' troughs (Figure 3a). However, when a peak of one wave interferes with an equal sized trough of another wave, destructive interference occurs, where the peak and trough cancel each other out[7]. This results in no wave at that specific point[7] (Figure 3b).

Thus, in Young's experiment, the interference pattern on the viewing screen implies that the massless particles of light—photons—are not the microscopic spheres that we may visualize them to be, but instead are waves[2]. After light passes through the two slits, the waves emanating from each slit interfere, and when this interfered wave interacts with the screen, it creates the light and dark bands we see on the screen[7]. When two peaks (or two troughs) interfere with each other and hit the screen, it makes the screen bright, resulting in the light bands[7]. When a peak and a trough interfere, the light waves cancel, resulting in the dark bands[7].

Figure 1

Experimental design of the double slit experiment. One can see the light, the blue sheet with the parallel slits, and the black viewing screen.

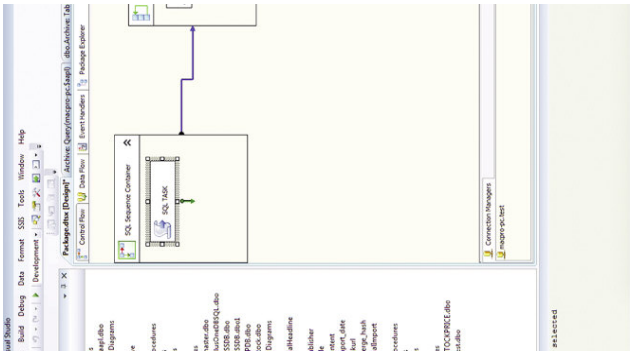

Figure 2

The viewing screen showing interference pattern resulting from conducting a double-slit experiment with a light.

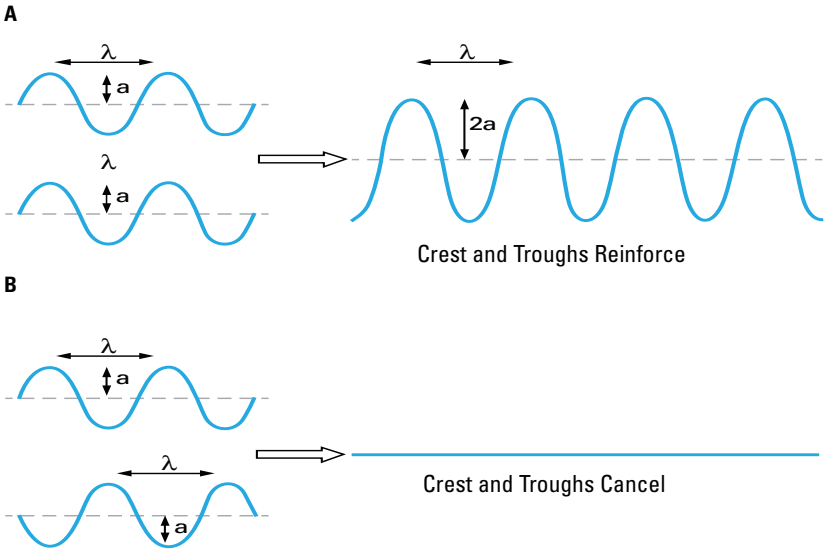

Figure 3

(a) Constructive interference: the peaks of the two waves combine to form a new peak that is equal to the sum of the two peaks of the original waves. The same occurs for two troughs.

(b) Destructive interference: peaks and troughs of equal height and depth interfere, canceling the wave.

An interesting paradox arises when one slows down this experiment using time-lapse photography (Figure 4). Ultimately in photo (e) the viewing screen displays the same interference pattern, indicating that light is a wave. However, in the earlier photos one can see that the interference pattern is created from particle-like impacts, indicating that light is a particle[2]. In other words, photons hit randomly like individual particles, but somehow when the random indi-

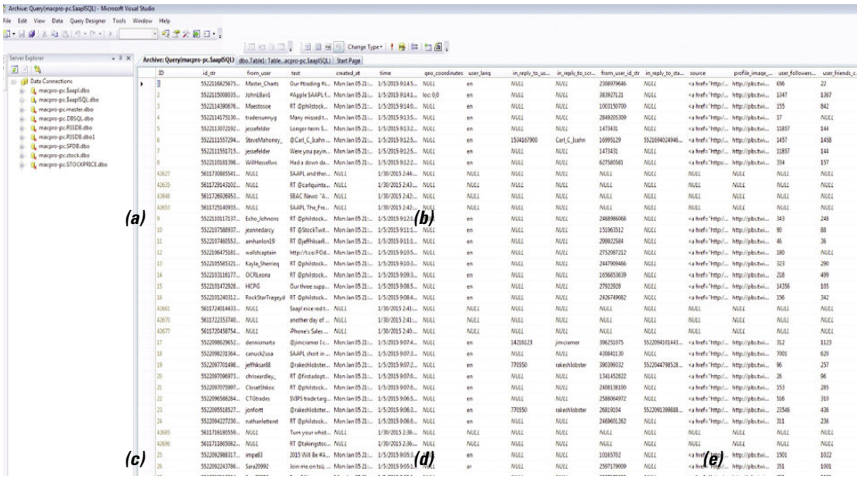

Figure 4

The viewing screen at different points over time using time-lapse photography, showing that while light seems to hit like particl es, once all particles have hit, it results in an interference pattern.

vidual impacts are added together the final product is a pattern that only results from waves. Because light appears to hold properties of both waves and particles, how can we resolve this paradox?

To answer this question, we must understand some things. The electromagnetic (EM) field is made up of "quanta"—bundles of energy that extend through all of space[4]. Imagine it as an invisible field, existing everywhere at once. A key feature of these quanta, or space filling bundles of energy, is that they are "discrete"—they can only have specific amounts of energy[4]. For example there could only be whole numbers of bundle of energy in the EM field, like 1, 2, or 3 bundles—never 1.3, 2.42, nor 3.6 bundles, or quanta. So there are no possible values between bundles—you could only go from 1 to 2 quanta, never from 1 to 1.1. Because of these restrictions, when this spatially extended quanta travel through space and interacts with other matter like a viewing screen, they must give an entire "quantum" of energy to the atom in the viewing screen[2]. Thus, the field quantum "collapses" instantaneously to atomic dimensions[4]. And that collapsed quanta is exactly what we see when we look at the viewing screen in Figure 4, photo (a). So, as seen from these experiments, photons are not tiny spheres emitted from the light source that move through space to later appear on the screen. Instead, a photon is a quanta: before it hits the screen, it is no tiny sphere with a specific location, but rather exists as quanta, or space filling bundles of energy, and only once this spread out bundle of energy hits the screen symmetrically does it collapse and thus hit like a particle[4] (Figure 5). Though the quantum hits like a particle, it is not one. Rather, a quantum is a spatially extended field that after collapse is spread out over atomic dimensions, causing it to appear to hit like a particle[4]. Photons are merely the particle-like bundles, or quanta, of EM energy[4]. As Steven Weinberg puts it, "quantum fields are the basic ingredients of the universe, and the particles are just bundles of energy and momentum of the fields"[5].

Another way to visualize these field quanta is as wave packets (Figure 6)[4]. The wave packet is a single quantum of EM field energy, and is the photon. Do not imagine that the photon is somewhere inside the wave packet.

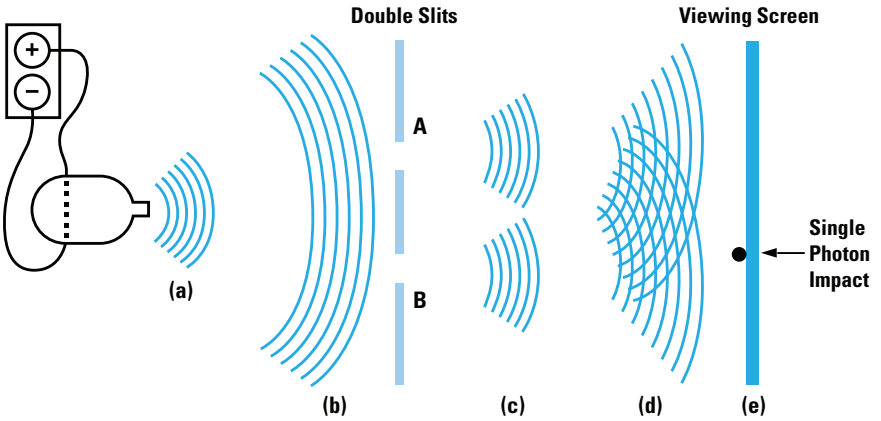

Figure 5

Double-slit experiment showing the EM field for a portion of a very low intensity photon beam, carrying a single quantum of EM energy. One can see how the extended EM field vanishes and collapses to atomic dimensions at the instant of impact.

Young's double-slit experiment was repeated but with one change: instead of shining a light beam, he shone an electron beam. The main difference between this and the first experiment is that while a photon is massless, an electron has mass. The results for this electron beam double-slit experiment were identical to the light beam double-slit experiment (Figures 7 and 8). Can a particle with mass really be a wave like the massless particle of light, known as photons? As it turns out, yes[2]. The logic is the same, but the specifics are different. Where in the case of photons we had the EM field, with electrons we have the "matter field"[2]. Like the EM field, the matter field's energy is discrete—meaning it is restricted to certain values (like 1 and 2, never 1.3)[2]. Thus, the same conclusion applies: an electron is not a tiny sphere, but rather is a quantum, or an energy increment of a spatially extended travelling field that when interacts with an atom on a viewing screen, collapses to atomic dimensions, causing the visible result of hitting like a particle[2]. In fact, all the particles of nature are the quanta of fields[2].

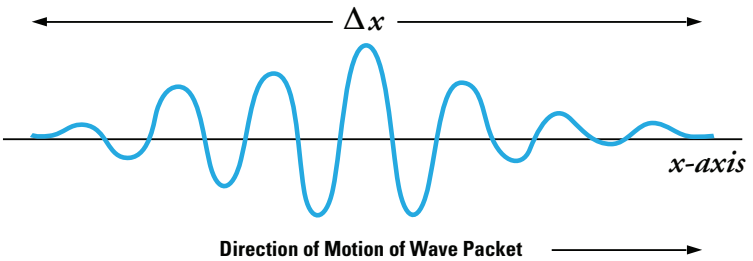

Figure 6

A wave packet for a single photon.

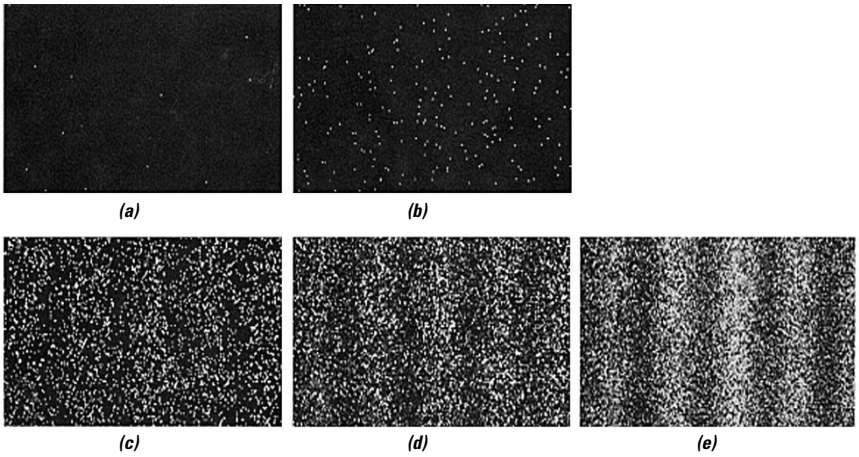

Figure 7

The viewing screen at different point in time using time-lapse photography, showing that while electrons seems to hit like particles, once all particles have hit, it results in the same interference pattern.

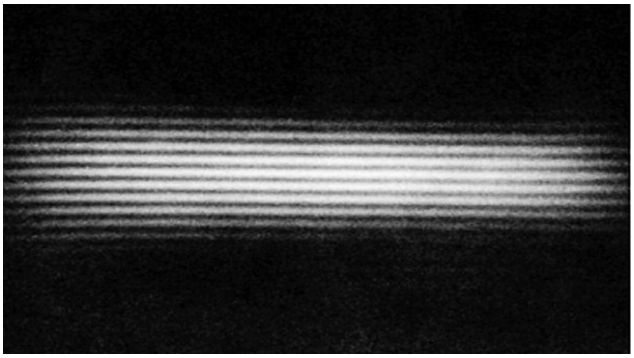

Figure 8

A photo of the viewing screen after the double-slit experiment was conducted with an electron beam. As one can see, the results are identical to that of the light beam.

Another aspect of a field quanta—which is representative of quantum mechanics in general—is their probabilistic nature. Classical, or macroscopic physics, conveys that full knowledge of initial conditions leads to fully predictable results. For example, when rolling dice, if we had all the necessary information of the initial condition, such as rolling strength, wind resistance etc. we would be able to predict with 100% accuracy the outcome of the die. If all the initial conditions were identical, the result of the die would never change. Thus, uncertainty or randomness in the macroscopic world results from incomplete information of the initial conditions[4]. However, the quantum world is different. In the double-slit experiment, all the initial conditions are known (from the laser). Yet, the electrons (or, rather, travelling waves that hit like particles) don't impact the screen at the same location—it appears that the impacts are random and scattered, and thus are unpredictable. One question remains: why do electrons (or any particles) impact the screen at different locations if the initial conditions for each were identical?

The answer to this questions lies in the symmetry of the field nature of electrons. When a field carrying one quantum of energy is traveling through space and hits the screen, it spreads out uniformly across the entire screen, hitting millions of atoms simultaneously[4]. However, the entire wave can only interact with one atom of the screen (because it holds only one quantum, one bundle of energy to give)[4]. Because the wave is uniform across the entire screen, it has an equal probability of interacting with each and every atom of the screen, rendering the actual particular atom that the field interacts with to be random[4]. And thus, this wave is actually a probability wave, extending across the entire universe[7]. When an electron beam is aimed at double-slits, after passing through the slits, it is the two probability waves that interfere creating the interference pattern found in Figures 2 and 8[7].

In this example, because the probability wave is uniform over the screen, the probability of the wave hitting any given atom on the screen is uniform. However, the probability wave, while extending through all of space, is not uniform over the entire universe[7]. This wave, because of certain conditions like being aimed at the screen, has a larger probability of being found over the screen—but this does not exclude the fact that the probability wave, though exponentially smaller, still extends throughout all of space[7]. Thus, although the possibility of this occurring is next to zero, given the spatially extended probability wave-particle nature of quantum mechanical particles such as electrons before they interact with other atoms, an electron's probability wave, while being aimed at a screen, could still collapse and be found even one million light years away[7]. Thus, in quantum mechanics individual events cannot be predicted with certainty: only the *possibility* of certain outcomes can be predicted[4].

This probability wave has another implication. In the classical world we experience, we expect that our observations are the results of physical properties that exist before we make the observation. We also expect that the outcome of our observations, which depend on pre-existing properties, are independent of the observation itself. For example, when we measure the length of a table, we expect that before we place the ruler along the edge, the table had a definite length and that measuring this length will not alter it in any way—we are merely measuring what already exists. However, the quantum world starkly contradicts this view of reality. Because before observation (before a wave hits the screen), waves exists as an entire space filling probability wave, particles *do not have a definite location before being measured*—before interacting with the screen[8]. They are everywhere and nowhere at once before being measured. The probability wave includes all of the possible locations simultaneously, and thus the locations are said to exist in "superposition", wherein all possible locations are described[9]. Only once an electron is detected does the probability wave collapse, giving the particle a more definite location. Thus, interacting with the screen in a sense creates the location of the particle[9].

From spatially-extended field quanta without pre-existing locations to inherent randomness, the world of the quantum acts very differently from that of the Classical one we observe. Now, armed with some of the fundamental knowledge of quantum mechanics, we are able to explore the details of two very interesting phenomena of quantum mechanics: entanglement and teleportation.

When a wave-like particle interacts with an atom on a screen, the interaction instantaneously transfers the particle's energy to a single atom of the screen[8]. Because quantized fields are discrete, they can only hold a whole number of "excitations" (particles like electrons or photons)[8]. However, a single field quantum can contain more than one excitation, for example two

excitations. When this occurs, any interaction of one excitation instantaneously affects the other excitation no matter the distance between the two because they are from the same single field quantum[8]. When two excitations exist like this in the same field quantum, they are said to be "entangled"[8].

Figure 9 is a way to picture the creation of and understand the meaning of entanglement. One can see the wave packets of Particles 1 and 2 (as a reminder, particles, or field quanta, are their wave packet) before interaction. Note that they are both one-particle, or one excitation, wave packets approaching each other. If they do interact (remember probabilistic nature), the two previously autonomous one-particle wave packets interact in such a way that entangles their wave packets, causing them to form a single two-particle wave packet for an indefinite amount of time[8]. This process cannot be reversed—one cannot separate the single two-particle wave packet into two separate one-particle wave packets[8]. The post-interaction packets (the top and right packets in Figure 9) are really two parts of the exact same packet, the exact same unified field quantum. This means that when one portion of the wave packet has any interaction (such as hitting a screen), instantaneously collapsing that portion of the two-particle wave packet, instantaneously the other particle in the wave packet collapses too because the entire field quanta collapses everywhere the instant one particle interacts[8]. Even if the two particles are light-years apart, they will both instantaneously be affected by the other and thus snap out of superposition instantaneously.

Not only does the entire field quantum collapse upon one particle's interaction, their properties, which, according to the randomness of quantum mechanics were in a wave of probability and thus should be completely random, are somehow perfectly correlated[8]. Particles have

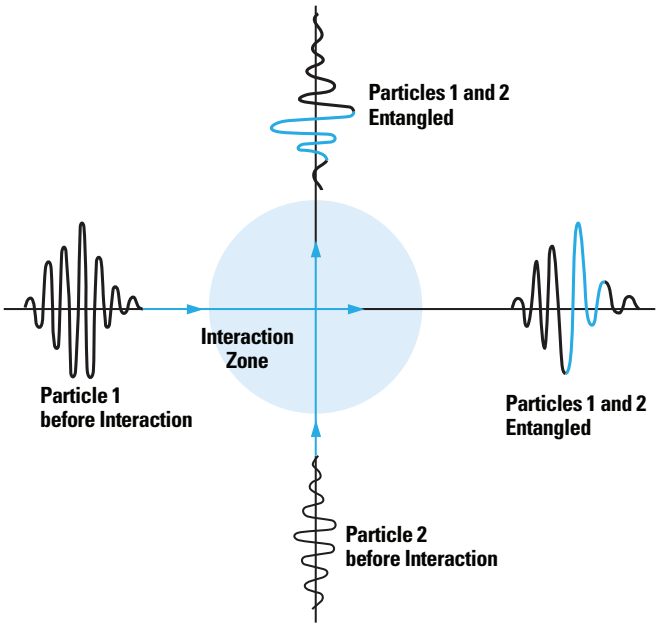

Figure 9

Displaying how entanglement occurs. Two particles' wave packets interacting in such a way that creates one, unified two-particle wave packet.

a property called "spin"; after measurement they can "spin" either up or down. Before measurement, however, remember that particles exist in a superposition of all their possible states. In the case of spin that means before measurement, the particles exist as a probabilistic combination of both being spin up and spin down at the same time. Suppose we have two particles, P1 and P2. Given the random nature of quantum mechanics, when measured, P1 and P2 would snap out of superposition and could be either spin up or down—there would be an independently random probability of either for each particle, meaning that P1 could be spin up, and P2 could be spin up or spin down—they would have no effect on each other. However, if these two particles were entangled, as soon as P1 was measured, let's say to be spin up, its probability wave would collapse, and since P1 and P2 share the same probability wave, P2 would also instantaneously be snapped out of superposition and have a spin of up.

To note, the entanglement relationship does not dictate that both will always be spin up. The entanglement relationship just states that they are informationally linked in some way—one pair of entangled particles could consistently both have identical spin values, but another pair could consistently have opposite spin values.

Because two entangled particles affect each other in a correlated way instantaneously no matter the distance between them, there are some applications of this phenomenon. Namely, physicists have achieved quantum teleportation[10]. However, this form of teleportation differs from the one you may imagine: no people will be teleported any time soon. Rather, as betrayed by its name, quantum teleportation deals with teleporting particles. Additionally, the process of teleportation does not include moving constituents from one location and recreating them at another location from pure air. Rather, quantum teleportation involves teleporting the *properties* of a particle from one location to another, and casting those properties onto a different particle to recreate the original[10]. But how does one achieve this teleportation?

Methods

Imagine that two scientists, Alice and Bob, are on the Earth and the Moon, respectively, and that they wish to teleport Photon 3 from Alice to Bob (Figure 10). And, as stated earlier, teleporting Photon 3 means teleporting its state, not the actual particle. In order to do so, they must share a pair of entangled particles. They also must have the ability to communicate classically —over the phone, for example. Additionally, a key feature of this process is that they can teleport Photon 3 with no prior knowledge of its properties[10].

To begin, Alice performs a simultaneous measurement on both Photon 3 and her entangled photon, Photon 1[10]. Because Photons 1 and 2 are entangled, when Alice performs a measurement on Photon 1, Photon 2 is instantaneously affected. In this case, Photon 2 is left in a residual state—one that is available to be transformed into Photon 3[10]. Additionally, as a result of Alice's measurement, the entanglement link is broken between Photons 1 and 2, and the original state of Photon 3—the one to be teleported—is destroyed[10]. From her measurement, Alice receives information that will allow Bob to recreate the state of Photon 3 in what was previously Photon 2[10]. Alice sends this information to Bob through classical means, and with this information, depending on what she sends, Bob performs a certain operation on Photon 2[10]. From this operation, the state of Photon 3 is successfully recreated in Photon 3, which was on the moon[10]. Teleportation splits the information in Photon 3 into two parts: the classical part,

Quantum Teleportation

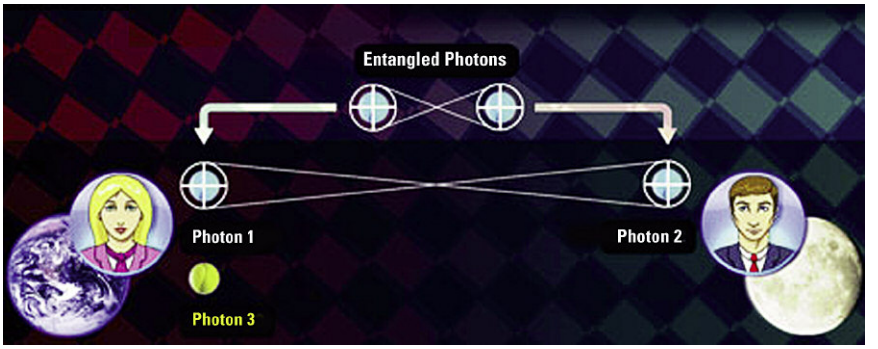

Figure 10

Teleportation design. Alice, on the left, wants to teleport Photon 3 to Bob, on the right on the moon.

which Alice communicates to Bob over the phone, and the quantum part, which is carried by the entanglement of Photons 1 and 2[10].

Conclusion

The applications of entanglement and teleportation are wide ranging. One application is the invention of quantum computers, which could be exponentially superior in speed and computational might to the computers we use today. Normal computers are binary: they speak in only 1s and 0s. Quantum computers, however, because of superposition, could speak in 1s, 0s, and the superpositions of 1 and 0. This means that quantum computers could factor much larger numbers than classical computers, which leads to another implication: cryptography. Cryptography—the art enciphering and deciphering codes—is based in the impossibility of factoring very large numbers: if a hacker can't factor the number, they can't break the code. When you shop online with your credit card, its information is encrypted this way. Quantum computers, however, with their computational power, could potentially factor these numbers and decrypt the code. However, with quantum computers' potential for decryption, comes quantum mechanics' potential for encryption: entangled particles are in effect a private quantum channel. An imagined third person in the above teleportation example could never intercept the full information because as soon as an attempt is made, because of entanglement causing the probability wave packet collapse of the entire wave, Alice and Bob would know if someone was trying to eavesdrop. A final application, perhaps one day, would be teleporting macroscopic objects.

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The Potential use of Synthetic Shark Skin as a Non-toxic Antifouler and Antimicrobial

by Niamh Micklewhite

Abstract

Biofouling is the presence of algae, barnacles, and mollusks on unwanted water submerged machinery. Their attachment causes reduced efficiency, contamination, and failure of equipment. The removal process, also known as antifouling, is usually done with a toxic chemical agent that can affect the surrounding ecosystem. Sharks have limited organism attachment due to the composition of their skin. Scientists have developed a synthetic version of shark skin for antifouling purposes that relies on physical composition rather than chemical. Scientists also found that it exhibited antimicrobial properties and that it could be used on medical devices and everyday surfaces. A study completed at Berkeley Carroll shows inconclusive results but does present synthetic shark skin as a potential antimicrobial.

Background

Every year, the Navy spends over 600 million dollars on fueling their ships and submarines; however, biofouling organisms [algae, hydroids, tubeworms, bivalve mollusks, bryozoans, and tunicates] can add up to 15 percent drag on a military ship/boat and cost the military up to 30 percent more money in fuel[7]. Biofouling is the growth of unwanted organisms on unwanted places. Biofilms or biofouling organisms[5] attach themselves onto water submerged pipes, machinery, fishing nets, or bases or hulls of boats, which causes reduced efficiency, contamination, and failure of certain equipment[2] (Figure 1). Biofouling removal is expensive, toxic, and the removal methods can decrease the efficiency and performance of the surface/machinery. In the Navy, the one billion dollars used to clean the hull yearly and the excess in fuel cost caused by the biofouling organisms could not only be prevented with an efficient antifouling device, but could also increase the overall speed at which the ships and submarines move[10,11].

The Navy, like many other international travel organizations, needs to think wisely about the toxins that they use for biofouling prevention and removal because of the range of waters that they travel in. The toxins in biocides have been present in waters all around the world, stretching from Japan, the United States, Singapore, Australia and Bermuda[4]. In New Zealand, scientists claim that biofouling from foreign waters has introduced other marine species into its waters, causing it to affect the native marine life[12].

When a surface or structure becomes biofouled, the structure gets heavier and can cause the it to corrode or malfunction. A surface that is submerged in the sea will eventually be

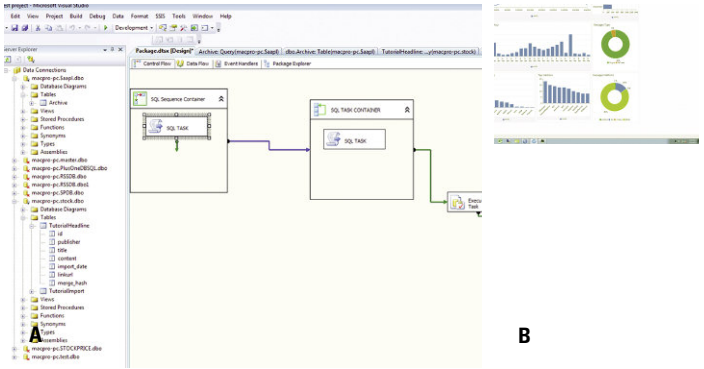


Figure 1

Examples of Biofouling

(a) Biofouling on hull of boat.
(b) Shows biofouling on a piece of water submerged equipment.

covered in some sort of organic material if left untouched[6]. In the fishing industry, biofouling organisms cover the fishing nets used to capture fish or other marine life. If the net is left with the attached biofouling organisms, it will not be able to supply enough oxygen to the contents inside the net[6]. This is an issue for two reasons: the contents of the net will and also waste fishermen's money and time. This is a moral and an economical issue.

Once the structure has become biofouled, the removal of the organisms is expensive, and this can cause a decrease in efficiency and performance of the structure to which the surface belongs. Usually, methods of removal are toxic to the surrounding water life. Therefore, it is better to prevent biofouling rather than remove it.

The removal, reduction, delay, or prevention of biofouling is known as antifouling coatings or antifouling strategies. The most commonly used antifouler is a biocide or metal compound[5] and many can be harmful to the surrounding ecosystems[7]. The removal process of biofouling organisms can be done mechanically (scrapers, brushes), chemically (acid), or with the use of a biocide, which, in this case, is a combination of chemicals that can kill certain marine organisms (algae, mollusks, barnacles, etc.)[5]. Sometimes, all three methods of removal need to be used because the hull or structure is biofouled so heavily.

After the biocide has killed all the biofouling organisms, the dead biofilms and organisms need to be removed because otherwise they will serve as the nutrients for the next generation of biofoulers to grow[8]. The biocide is not efficient enough to prevent any further attachment and so the cycle begins again. The dead organisms need to be removed and then an antifouling coating needs to be placed onto the newly cleaned surface[8]. The most commonly used antifouler is TBT, which is short for tributyltin[4]. TBT usually comes in the form of paint and is applied before exposure to seawater, as it is used for the prevention of biofouling. International regulations and rules against the use of TBT have lessened its use but have made the use of copper more common[5].

The sea urchin has shown to be affected by biofouling removers[13]. A study conducted by Kobayashi et. al. tested biocides and saw that sea urchin eggs and embryos were affected[13]. The study concluded that depending on the biocide and concentration of the biocide in the water, the developmental rate of the eggs/embryos would change dramatically[13]. One of the biocides tested was TBT.

Finding a Non-toxic Antifouling Solution

The goal for scientists concerned with biofouling is to find an antifouling solution that will not be harmful to the environment. If a safe, non-toxic, environmentally friendly antifouling coating or surface is found, harmful biocides and chemicals would not be used as frequently and perhaps diminish the drag and fuel cost of a ship/boat, as well as limit the negative effects on the environment. The most effective antifouling device would consider both the physical and chemical composition of the surface[1].

So far, there are limited eco-friendly antifouling solutions. Instead of using a type of eco-friendly, non-toxic chemical or cleaning product, scientists are trying to change the composition and surface structure of the water submerged equipment[5]. Scientists have tried placing another surface on top of a hull a boat, such as one that is super-hydrophobic[9]. Scientists believe that if the hull does not come in contact with the water, then the biofouling organisms will not have contact with the hull and therefore not be able to attach[2]. Scientists are looking at the natural antifouling mechanisms of sharks, mussels, seaweed and crabs because each have their own way of preventing organism attachment[1]. Many of the new antifouling strategies are structures where the surfaces are mimicked from a surface that is naturally occurring.

Synthetic Shark Skin as an Antifouler

As previously mentioned, there are structures that occur in nature that have antifouling properties based on the formation, texture, and shape of its surface. Shark skin has natural antifouling properties, which allows sharks to swim silently through the water with minimal drag. Sharks are predators and never stop moving, so their survival is dependent on their hunting abilities. Interestingly, sharks are one of the oldest creatures on earth, so there must be something about their design that has allowed them to survive and thrive.

Unlike a whale, when a shark swims through the water, it does not have biofouling organisms trying to attach to the surface of its skin. This is because of the simple geometric shape of the shark's dermal denticles; dentine and enamel scale like structures. These structures look like teeth or scales and feel like velvet, depending on the direction of touch

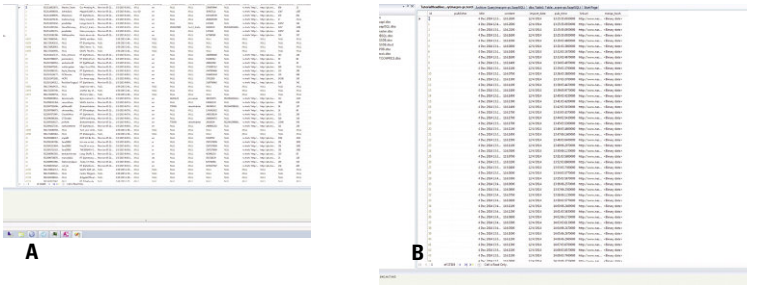


Figure 2

Images of Shark Skin and Synthetic Shark Skin

(a) Scanning Electron Microscope image of scale-like dermal denticles. Shows the scales of the dermal denticles but not the small ridge-like structures.

(b) Close up of Sharklet showing the diamond ridges.

(Figure 2). The shark's body is covered in the scales ranging in size and shape. On the denticles, there are small ridges that act as a secondary guard against organism attachment.

In a study conducted in 2011 by Sullivan and Reagan, specimens of catsharks were collected as a dead bycatch in lobster traps from fishing boats near Baltimore Harbour, County Cork, Ireland[14]. Adult sharks that were roughly the same length were placed into a cool box and transported to a lab[14]. At the lab, skin samples of 1 centimeter squared were taken from the specimens and examined under a Scanning Electron Microscope (SEM)[14]. Using the SEM, 150 dermal denticles were taken from four locations on the sharks' bodies (head, first dorsal fin, second dorsal fin, and caudal fin)[14](Figure 3). From the four locations, the skin was replicated with poly elastomer (PDMSe), a type of rubber or plastic, in a mould[14]. The synthetic skin was

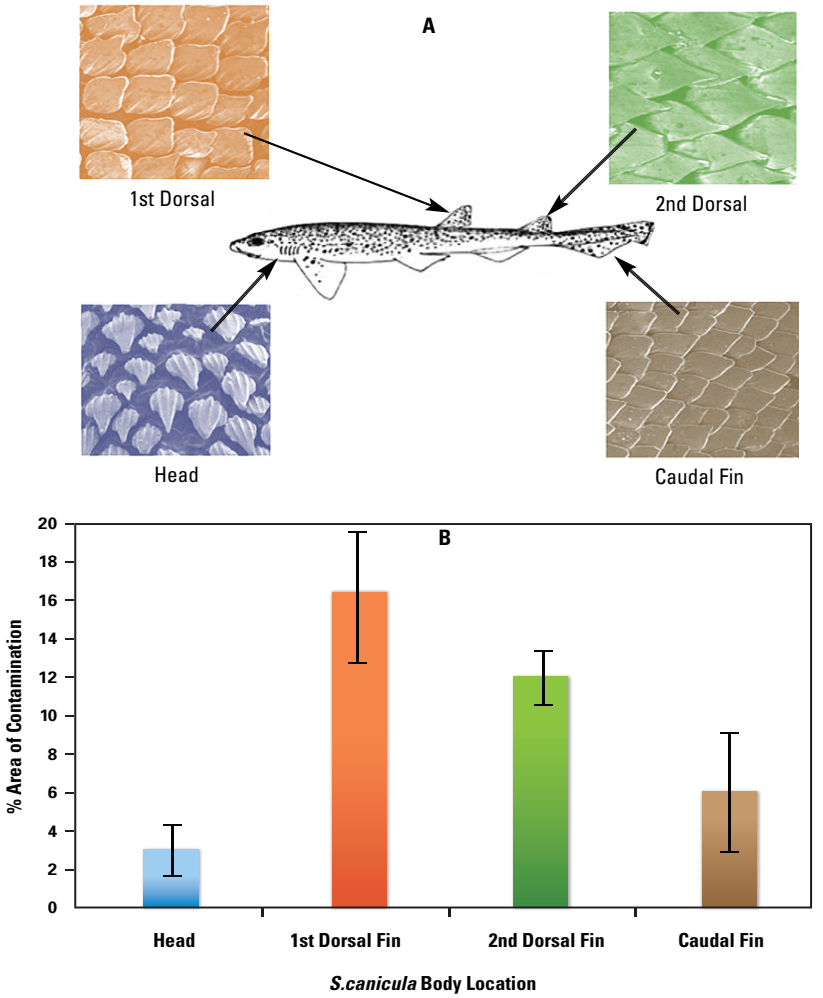


Figure 3

Sullivan and Reagan Study

(a) Dermal denticles of shark differentiate in length, width, flatness depending on the placement on the shark's body.

(b) Results of study show that the percent area of contamination is heaviest on the 1st dorsal fin and the least on the head. The scale shapes are very different.

then placed in real life conditions consisting of high and low flow (amount of organisms and force of water) fouling conditions for 14 days[14]. The placement of the synthetically made shark skin allowed for scientists to show the potential use of the shark's dermal denticles in the water but off the shark[14]. However, the scale shape, size, and texture changed depending on the location on the shark's body (Figure 3a). Sullivan and Reagan concluded that there was less organism attachment towards the dermal denticles approaching the head of the catshark, where the denticles were rounder (Figure 3). The percent area of contamination along the four sample locations of the shark differ
(Figure 3b).

The study concluded that the use of synthetic shark skin in the boating and marine industries has the potential to be a non-toxic antifouling device. The actual prevention mechanism of the biofouling organisms is the surface texture and physical composition, not the chemical composition of the surface. Both the smooth surface and the sharklet were replicated out of PDMSe, which has no antifouling properties. Because of the nature of shark skin in the water and its ability to reduce contamination, scientists saw the possibility of the surface having antimicrobial prospects.

Use of Synthetic Shark Skin for Antimicrobial Purposes

The idea of synthetically made shark skin evolved beyond the marine engineering industry. Taking the idea that synthetic shark skin is an effective antifouler in the water, bioengineers and biomimeticists started to engineer the surface for real life applications, such as biomedical surfaces (urinary catheters, pacemaker wires) and high touch zones (doorknobs, faucets).

The surface of a high touch zone can be colonized by human pathogens that can be harmful to human health. There is a need for a surface that will help to minimize these biofilms, which are structured communities of microbial cells[15]. Preventing these biofilms has usually been through antibacterial agents but that practice has contributed to the evolution and development of antibacterial resistant bacteria. A surface that prevents bacterial attachment and bacterial growth would limit the use of antibacterial agents[15].

A surface derived from nature that takes into account natural capabilities is Sharklet, a form of synthetically made shark skin. Sharklet is a non-toxic and biocide-free surface pattern used to inhibit bacterial attachment, survival, migration and touch-transference[16]. Sharklet is used in places like hospitals to reduce the chance of spreading infections[16]. Sharklet is composed of ribbed diamond structures; each diamond is about 1/5th the width of an average human hair[16] (Figure 2b). When manufactured, the pattern cannot be seen with the naked eye or felt to the touch[16]. Sharklet Technologies does not release the material that their surface protectors are made out of, but a study conducted by Chung et al tested the textured surface. This was to investigate the potential for bacterial attachment and colonization on an engineered topography with a well defined structure[15].

The structures used were Sharklet's engineered devices, and they were replicated using the same material as the smooth surface[15]. The Sharklet continually had less percentage of area covered (Figure 4). The bacteria on the smooth surface also increased quicker, showing that the bacteria on the smooth grew faster. After 21 days, the replicated Sharklet surface was only 35% covered with biofilms compared to 77% for the smooth surface[15] (Figure 4b). This is a significant difference in bacterial coverage.

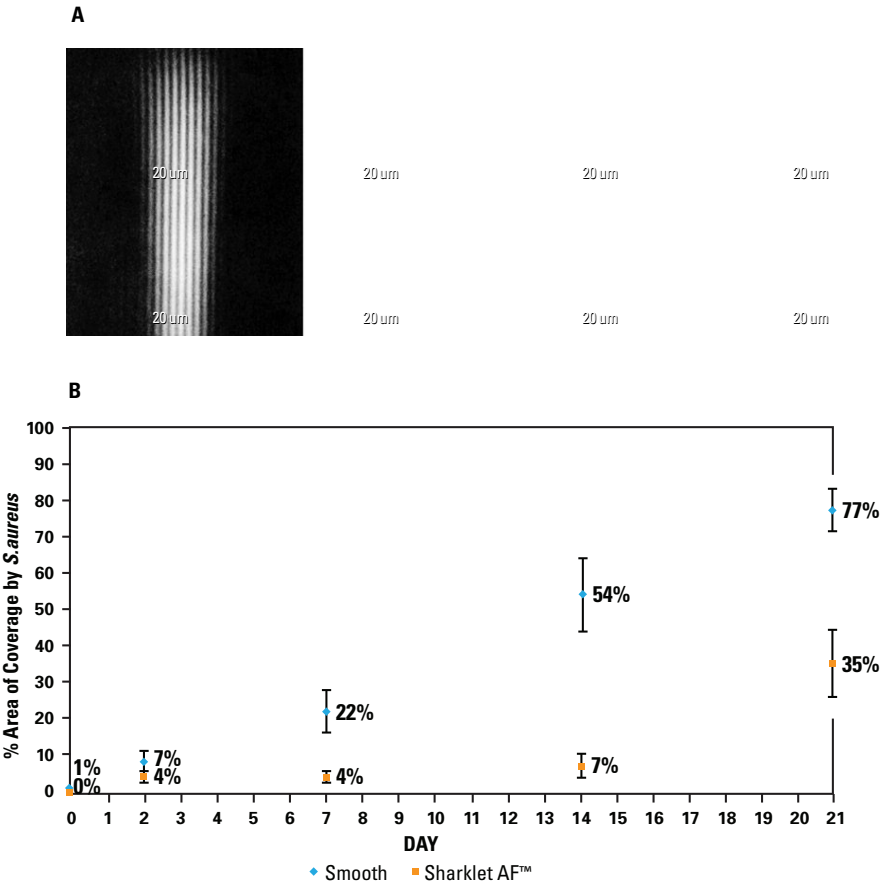


Figure 4

Results of Chung et al

(a) Growth of S.aureus *bacteria over time (day 0, 7, 14, 21) between smooth and sharklet.*

(b) Percent area of coverage by S.aureus *over time.*

Both the experimental and control surfaces were made out of the same material, which demonstrates that the texture inhibits bacterial growth, not the material. Between the two compared surfaces, the only difference was the patterned riblets on the Sharklet surface. This meant to demonstrate that the pattern on the surface contributed to the bacterial growth or inhibition of growth. Chung's study suggests that bacteria and biofilms would have a significantly lesser quantity on the sharklet surface protector than the smooth surface. One explanation for this result is that bacteria are social creatures, and the riblets of sharklet prevent the bacteria from replicating beyond their capabilities.

First Study Conducted at Berkeley Carroll

The objective of the first study was to compare the bacteria quantity on Sharklet and a smooth surface, using the bacteria *Staphylococcus epidermidis.* This bacteria is not harmful to humans as it found on human hands. *S. epidermidis* bacteria streak was resuspended in 5 mL of sterile PBS (Phosphate Buffered Saline) and pipetted into centrifuge tubes. After centrifugation, the

supernatant was discarded and the pellet of bacteria was massed. The pellet was then resuspended in 5 mL of PBS. This volume was then pipetted onto the control and Sharklet surfaces in petri dishes, and the surfaces were incubated at 374C for 7 days.

An initial mass for the surface and bacteria was recorded, as well as a final mass after the 7 day incubation period. Percent change in mass would then be calculated. While it was expected that there would be a change in mass, the experimental design did not account for the minimum nutritional requirement for relative bacteria growth. A second study was needed to address this limitation. This study was therefore inconclusive.

Second Study Conducted At Berkeley Carroll

To improve the first experiment and incorporate the bacteria's minimum nutrient requirement, a different approach was taken to testing the smooth and sharklet surfaces. Sharklet was placed on a door plate and water fountain handles around Berkeley Carroll. Any water fountain could have been used, but the door had to have a flat plate so the test surfaces would have equal amount of hand exposure, as otherwise uneven surface could potentially have resulted in

Figure 5

Locations Tested Around Berkeley Carroll

(a) The right-side door.

(b) Side of water fountain.

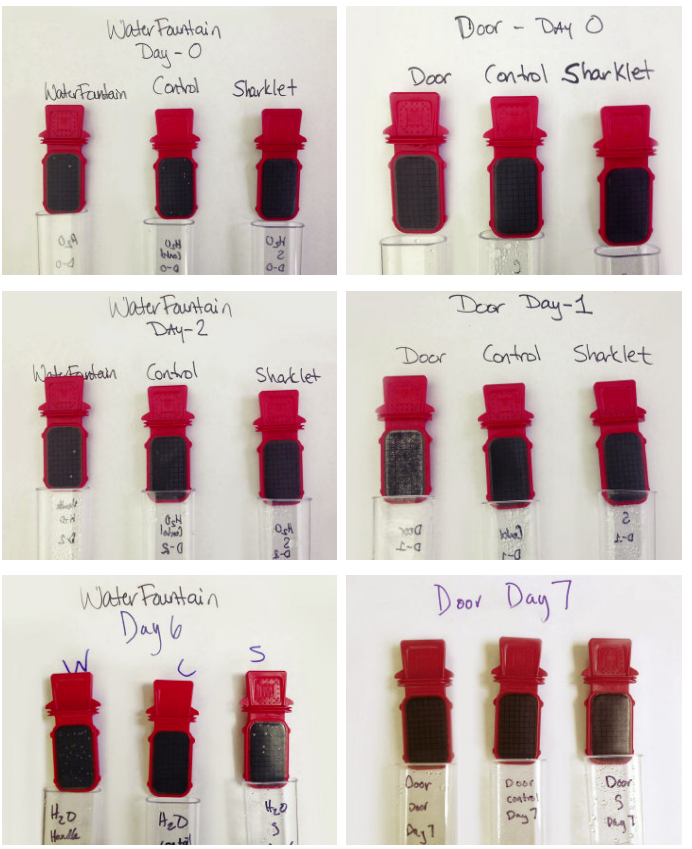
(c) Front of water fountain.

skewed and inaccurate data (controlling for external variables) (Figure 5). If the Sharklet samples show to have a decrease in colony forming units (CFU) count than the smooth surface, the study would suggest that synthetic shark skin can reduce the potential for bacterial replication and for sickness outbreak inside Berkeley Carroll.

HPC Samplers were used to obtain the number of colony forming units in 1 milliliter of water. The gridded, millipore, absorbent pad contains dehydrated nutrient medium for the bacteria to grow but not thrive (Figure 6b). This ensures that the bacteria are growing at the correct stage of the bacterial growth curve, where the bacteria can barely survive. These samplers were imperative to the quantification process of the bacteria growth on the surfaces.

The two surfaces, Sharklet and smooth, were cut into thin strips approximately 8 by 3 centimeters and were then placed next to each other so that each surface was exposed to the same amount of human contact. On the door, the surfaces were placed in three locations, as seen in figure 5. Three surfaces on the door were tested: Sharklet (Experimental), smooth

A



B

Figure 6

HPC Sampler Bacteria Colonies

(a) Swab cultures from water fountain and door over day 0 to day 6/7 in comparison to each other.

(b) The HPC sampler before use.

(Control^{+}), and the door (Control^{-}). Control negative is the actual surface, while control positive is a smooth surface. Similarly, on the water fountain, the surfaces were placed close to each other and placed in three different locations. On day 0 (placement day) of both the door and water fountain studies, a sterile swab was taken and brushed along one surface on all three locations in an 'M' like pattern. The swab was then placed back into its solution filled container and given thirty shakes. Then, the swab was removed and the handle of the HPC Sampler was inserted into the solution and left without movement for thirty seconds; this is where the 1 milliliter of liquid is collected into the gridded pad. After the allotted time, the sampler was then placed back into the holder and put into the incubator for 5-7 days until the CFU were visible enough to be counted.

This methodology was repeated using different HPC samplers and sterile swabs for each surface on days 1, 3, and 7 for the door and days 2 and 6 for the water fountain (see figure 6a). All the HPC Samplers were left in the incubator for 5-7 days until the CFU were visible enough to be counted.

Table 1 and Table 2 show the data collected from the study. Only one trial was completed, so statistical conclusions cannot be drawn. On the door, both the Sharklet and the smooth surface exhibited the same number of CFU on day 0 and on day 7. The water fountain showed much more drastic results. Sharklet (Experimental) had a CFU count of 10 on Day 6 compared to the water fountain's 41 (Figure 7). Interestingly however, Sharklet displayed more CFU than the smooth surface. For the three experimental groups, the number CFU increased the most by Day 6, but it seems that the slowest growth came from the smooth surface (Figure 7).

Table 1

Colonies Counted on Door Swabs

	Day 0 (in CFU)	Day 1 (in CFU)	Day 3 (in CFU)	Day 7 (in CFU)
Experimental	0	0	Dried*	1
Control Negative	0	0	Dried*	0
Control Positive	0	Dried*	1	1

**Unable to collect data because the HPC Sampler dried up.*

Table 2

Colonies Counted on Water Fountain Swabs

	Day 0 (in CFU)	Day 2 (in CFU)	Day 6 (in CFU)
Experimental	0	0	10
Control Negative	9	1	2
Control Positive	3	3	41

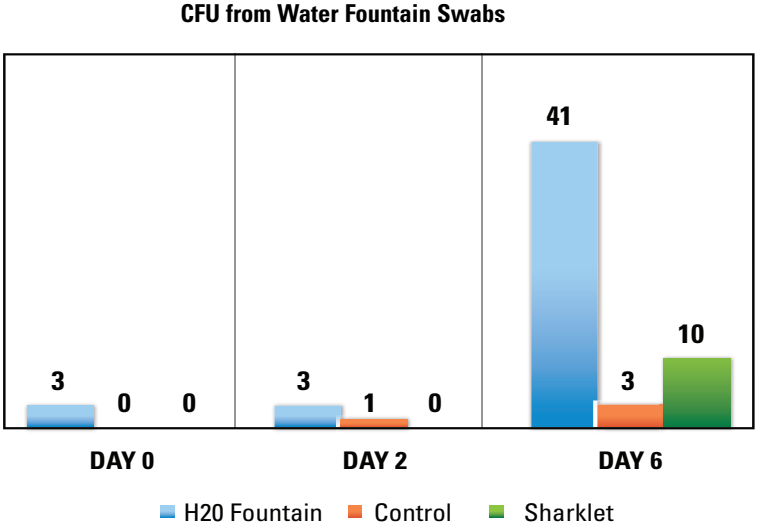


Figure 7

Graph of Results (Table 2)

Conclusions/Discussion

It is interesting that the control surfaces, the smooth, did not have as much bacterial growth as the Sharklet or the actual surface. The hypothesis, that the Sharklet would prevent bacterial growth more than the smooth, is not supported by these results. However, in the case of bacterial prevention, Sharklet exhibited a significant difference in CFU count than the actual surface.

Unfortunately, on day 1 and day 3 of the door study, the HPC sampler dried up. This was obvious by the light gray revealed on the gridded pad when usually the pads were dark gray after they had been exposed to the bacteria swab water. Another difficulty of this study was figuring out where people touched the water fountain and door. As it was winter at the time of this experiment, many people seemed to brush their winter jackets along the door to open it or open it with their bodies, instead of using their hands, as was expected. This could have skewed results, but it also could have made the study more realistic; the conditions were real life. This being said, it would be interesting to complete the same study during the spring. More skin to surface contact would be expected under these conditions, and the warmer weather might increase the amount of bacteria present on the surfaces.

Also, in an ideal world, the surfaces would have taken up the whole door. This would reduce the uncertainty of the locations people touch on the door. It was a little easier to find the area of the water fountain handle people were touching because the surface area was smaller. It is also important to note that the door and water fountain are completely different texture and material. It would be interesting to do a similar study on two surfaces that are the same material and texture but also compare the CFU count to sharklet and smooth.

The second study was inconclusive because only one trial was conducted. However, I do think that some conclusions can be made about the surfaces. In both instances (door and water fountain), the experimental surface exhibited less bacterial growth that the control positive. I would need more trials to say for certain whether or not sharklet works as an antimicrobial, but

I can say that Sharklet has shown to decrease bacteria quantities in comparison to a normal surface and therefore should be implemented by Berkeley Carroll.

I think that this study can benefit Berkeley Carroll and perhaps change the way the school views cleaning. In the short run, less bacteria in circulation would possibly cause fewer colds and sickness outbreaks. Students would miss less school, and the teacher and student population would be stronger healthwise. I also think that the student population would like to know that they are in a bacteria safe environment. However, this being said, in the very long run, the humans of Berkeley Carroll might not build up resistance against everyday bacteria, causing more sickness towards bacteria that once did not harm us. So, I think that there is a fine line between how much is too much. I suggest that synthetic shark skin be placed only on high touch surfaces, such as bathroom stalls, water fountains, and on bathroom water taps. This way, Berkeley Carroll is limiting the bacterial population but also not completely diminishing it.

In the future, I would like to conduct more trials of the second study and also test the efficiency of Sharklet mixed with another cleaning agent. This way, bacteria have an even less likelihood of replicating and spreading. But like I said, I think I would limit the use of both methods combined to only certain locations around the school.

In general, there is much more to learn about sharks. Companies like Sharklet and Speedo (who designed the 2012 Olympic shark skin swimsuits) have tried to mimic shark characteristics. Sharks have been swimming the waters of earth and have been able to survive for millions of years using virtually the same design. Sharks never stop swimming: if they do, they die. Perhaps this ability, or drawback, adds to their biofouling mechanisms, but this is also something that Sharklet will not be able to redesign. Will synthetic shark skin ever reach the capabilities of real sharks and shark skin?

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Figure 2b: http://www.asknature.org/images/uploads/product/3c21a56f0ba2ae-549894a2bf79372bda/45degreesharklet.jpg

Figure 2a, 3: Sullivan, Timothy, and Fiona Regan. "The Characterization Replication and Testing of Dermal Denticles of Scyliorhinus Canicula for Physical Mechanisms of Biofouling Prevention." Bioinspiration & Biomimetics (2011): n. pag. IOP Publishing Inc. Web. 19 Oct. 2012.

Figure 4: Chung, Kenneth K., James F. Schumacher, Edith M. Sampson, Robert A. Burne, Patrick J. Antonelli, and Anthony B. Brennan. "Impact of Engineered Surface Micro Topography on Biofilm Formation of S. Aureus." Biointerphases 2.2 (2007): 89-94. Web. 7 Oct. 2013.

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Heroin Self-Administration in Rats

by Samantha Schreiber

Abstract

What are the effects of heroin self-administration in rats and how can that be applied to teenage usage? It is understood that when prompted a little, rats will self-administer heroin. In order to accomplish this, rats were hooked up to a syringe that was connected to a catheter in the back of their necks. They were placed in a self-administration chamber, which had two levers: an active and an inactive lever. When pressed, one lever would dispense heroin to the rats and the other would provide nothing to the rat. It was discovered that most of the rats in the experimental group continued to self-administer the heroin when given the opportunity, even if the circumstances changed, such as an increase in the number of lever presses before heroin was received.

This research is important because it is very applicable to today's society. Studying the effects of heroin self-administration on rats can be applied to teen usage and is something that could greatly benefit people in the future. Perhaps there is a reason that teenagers are self-administering heroin, which could then be targeted to stop such occurrences from continuing.

Studying the brains of the rats after their session of self-administration was a different field of study, which means that the effects of heroin self-administration on the brain (which could be applied to teenagers) is unknown to the researchers working directly with the rats. Perhaps testing different variables such as rats in solitude v. rats with families (as tested before with different drugs) could provide more insight into the field of heroin self-administration.

Introduction

Addiction is based on memory and repetition. It occurs over time with training and prompting. It is difficult to tell when something becomes addictive if a third party is doing the administration because there is always someone else administering the drug—it is not the choice of the drug recipient. Addiction becomes much more apparent though, when it is being self-administered. Heroin self-administration has been performed on rats for both short and longitudinal studies. I took part in a three week study looking at the effects of heroin self-administration in rats. Rats were provided with two levers: an active and an inactive lever. The active lever would provide heroin (in the experimental group) or saline (in the control group) and the inactive lever would provide nothing to the rats. In the beginning, a rat wouldn't know the difference between the active and inactive levers, so they would experiment and both levers would have spiked results; but as the time progresses and the rats experience the addictive quality of heroin, they pressed the active lever more. According to many studies on this

subject—such as *Parental THC Exposure Leads to Compulsive Heroin-Seeking and Altered Striatal Synaptic Plasticity in the Subsequent Generation*[1], by Dr. Yasmin Hurd, among many others—if a rat displays similar results with elevated active lever presses for three consecutive days, then they are what can be considered addicted to heroin.

Self-administration is very difficult to work with in rats. On one hand, that is really the only efficient way to see if an animal is actually addicted to the heroin. On the other hand, a rat may not learn the difference between the levers or simply may not care. Although it is difficult to use self-administration in rats, it is still the most effective way to emulate the effects of heroin on teenage users[2]. The subject is receiving heroin by its own free will and eventually becomes addicted.

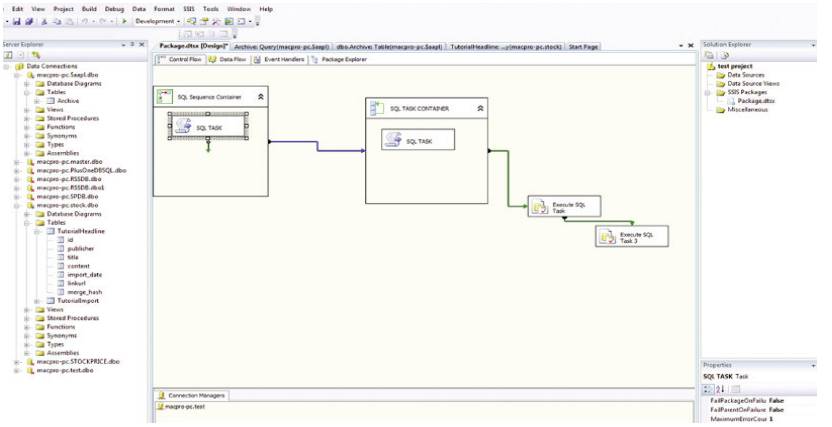
It is quite difficult to tell when a rat has become addicted, but according to Veronique Deroche-Gamonet and associates[3], there are three criterion for human addicts that can be also be used to tell if rats are addicted to heroin. These three addictive behaviors are: 1) trouble limiting and/or stopping drug usage, 2) highly motivated to take drug—willing to put in work to receive the drug, and 3) continued use despite harmful outcomes (a shock on the feet for rats). According to Deroche-Gamonet et.al.—who experimented with rats—a rat is considered addicted when it scores in the 66th-99th percentile for one of these three behaviors. This score was based on how often and how well they demonstrated these listed behaviors in different conditions. The rats were given an extinction period (to see how they react to a withdrawal of the drug), a higher FR-value (more lever presses required to receive the same amount of drug), and a shock on the feet when they pressed the lever (to see if they would continue to press the lever when provided with negative reinforcement). 17% of rats scored in the 66th-99th percentile for all three criterions, which is similar to the 15% of humans to score highly for all three behaviors. They also stated that it is believed that 90% of all addicts, in general, relapse, so the rats were put on a drug reinstatement program, where after a period of drug extinction, they are reintroduced to the drug. In most experiments with a drug extinction period, the drug is considered extinct when the rat has broken the trend of lever presses previously demonstrated, for three consecutive days. If a rat relapses after an extinction period, they are most-likely addicted to the drug: as long as this criteria is the same for rats as it is for humans, which is most likely true, considering the three behaviors suggesting addiction.

"Drugs reinforce the behavior that results in their delivery" (Panlilio and Goldberg)[4]. This quote perfectly correlates to self administration studies done in the Hurd Lab. In our case, pressing the lever results in the delivery of the heroin, therefore the more drugs they take in, the more likely they are to press the lever. Self-administration paired with addiction creates a never-ending cycle for the rats. This best mimics what adolescents experience when taking heroin. They go through the same process, from where they buy the heroin, to where and how they inject it, they become just as addicted to the habit of purchasing and injecting the heroin as they do to the actual heroin itself. Every time they see the person they buy it from or hear certain sounds, they get a rush of dopamine because all of these stimulants are now affiliated with the drug itself. For the rats, once addicted, the handling and weighing, being placed in a chamber attached to a syringe, the lever and even the light that turns on when the active lever is pushed, all release dopamine in the rats' brains because they are all associated with receiving the heroin. Habit is half of the addiction. It is just as hard to break the habit surrounding taking heroin as it is actually breaking away from the drug. The actions and behaviors leading up to receiving an addictive drug can be just as addictive as the actual drug itself.

Materials

In our experiment, we used very specific materials in order to best emulate the self-administration conditions of humans, in the rats. Our materials included:

- self-administration chambers
  - boxes with levers for the rats to press and a syringe that connects to the catheter on the rats back
  - levers: active (leads to dispension of heroin or saline) and inactive (nothing happens when pressed)



(This is an example of the type of self-administration chamber used, including all aspects except the Olfactory cue, which would cater to the rats sense of smell)[5]

- the boxes were connected to the computer, so each time a lever was pressed, it would automatically get recorded in the computer
  - syringe has either saline (control group) or heroin (experimental group) that is injected into the rats when the active levers are pressed
  - food pellets as a reward → dispensed in between the two levers
  - a blue light that goes off when the rats receive heroin
- 20 Long-Evans rats → 10 control and 10 experimental
- catheters that are in the right jugular vein of each rat
- heparin → injected into the rat before being placed in the chamber → prevents the catheters from clogging up
- food (usually 15 grams)
- red light[6] → rats cannot see red light so it is used during the experiment in the chamber because rats are on the dark part of their light/dark cycle then → the dark portion would be when they are active because they are nocturnal
- Computer: the computer records all of the data collected in each of the chambers
  - a normal Dell computer with an application that made the chambers function
  - the computers are what activated the chambers and allowed the heroin or saline to be dispensed→ the two were linked together

Procedure

The procedure was very monotonous in order to ensure that the rats would develop a set routine leading up to receiving the heroin. Each day we would bring rats into the room with a blanket over the cages because they were in the dark portion of light/dark phase[7]. Rats cannot see red light, so that is what we used in the room during the dark phase of the cycle. They would go through a light/dark cycle because rats are nocturnal so this would ensure that the rats would be awake and active during the dark phase—when they were placed in the self-administration chambers. For twelve hours each day, the rats would be placed in the light, where they were expected to sleep, and for twelve hours the rats were placed in the dark, where they were expected to be active because they are nocturnal creatures. There was also a loud fan blowing the entire time to ensure that the rats would not be affected by any outside noises, such as putting food in their cages or the slamming of the door when people entered and exited the room.

First, we would take each rat out of the cage, weigh it, and record the weight on a chart. Then we injected heparin, an anticoagulant, into the rats to avoid their catheters getting clogged. This ensures that the rat would receive any of the administered drug. Next, the rats would be placed in their specified chamber. Each rat was assigned a specific number (1-20) that was written on their cage. There was then a corresponding self-administration chamber, so that the rat would always be placed in the same chamber. This was necessary in order to maintain a controlled environment and ensure that the rat would be exposed to as few differences as possible, leading up to their receiving heroin. We closed the doors and turned on the chambers using the computer that also records all of the data collected in each chamber. We would then go to a second room and repeat that process. There were two rooms with ten rats in each of them.

The rats were then left alone in the self-administration chambers for three hours. During this time, 15 grams of food would be placed in each cage, so the rats would have plenty of food when they finished their testing for the day.

After three hours, the rats were taken out and returned to their cages. They were always really hungry afterwards, so putting the 15 grams of food in beforehand was crucial. Once all the rats were returned to their cages and brought back down to the animal storage room, all of the cages had to be cleaned. On a given day, two three hour sessions with two different groups of 20 rats was normally performed. To make sure that the scent of the previous rat or its food would not affect the results of the next rat, the cages had to be completely wiped down from top to bottom. This was the case, five days a week, for three weeks.

Data

Figures 1 and 2 demonstrate the active and inactive lever presses on the first and last days of the experiment. Rats 1-10 are the experimental group and Rats 11-20 are the control group. Each time a rat tapped on a lever, the press was recorded on the computer that was attached to the self-administration chambers (boxes). The before and after bars are displayed next to each other so that the change in the number of lever presses, for both the active and inactive levers, can easily be understood.

Figure 1

Number of Active Lever Presses on First and Last Day of Experiment

Figure 2

Number of Inactive Lever Presses on First and Last Day of Experiment

Figure 3

Total Lever Presses on the First Day and Last Day of the Experiment

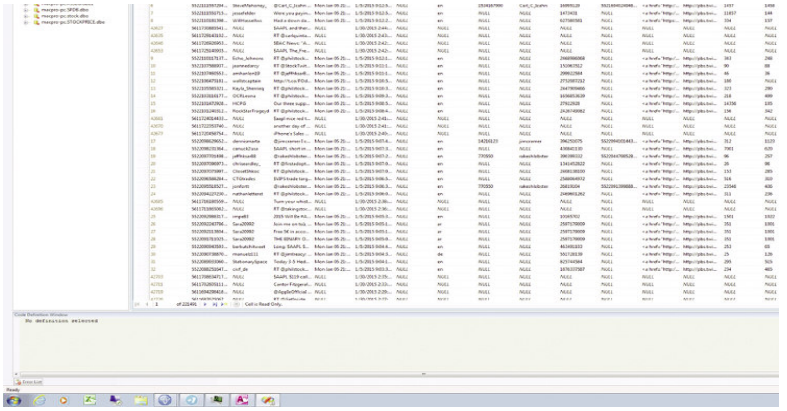


Figure 3 shows the overall lever presses on the first and last days of the experiment. This demonstrates the behavior of the rats very effectively because it can be seen when the rats pressed the levers more—before or after they had an idea of what the levers did for them.

One variable that is not displayed in these figures but is worth noting, is FR-value. An FR-value controls the number of lever presses required to receive a reward—in this case it would be heroin. In the first half of the experiment, the FR-value was at 1, which means that one lever press = one dosage of heroin. For the second half of the experiment, the FR-value was bumped up to five. This means that five lever presses = one dosage of heroin. The dosage of heroin remained constant throughout the experiment, the only changing variable was the number of times that the rat pressed the lever.

Results

Figures 1 and 2 show the results for the twenty rats that were tested, on the first and last days of the experiment. The number of both active and inactive lever presses for all twenty rats varied greatly due to the fact that the rats were really just experimenting with the levers. Some rats were only pressing each of the levers 0-2 times in a three-hour session. There may be many explanations for that, like a blocked catheter or disinterest because they were not yet exposed enough to the heroin. Either way, the rats were not receiving the right amount of exposure in order to become addicted to heroin. They were not sure what lever led to the "reward" or even what that "reward" was. There are ways to prompt rats to push the levers more, for example: auto-shaping. Auto-shaping is when the researcher interferes to get the results that they want. The researchers would do this by sprinkling rat food powder on the active lever. This would prompt the rat to press that lever and therefore at least be exposed to the drug once. Once the researchers could be sure that the rat experienced the heroin at least once, then they

would stop auto-shaping. A rat like number three in figure 1 would be auto-shaped.

Figure 3 is a comparison of the total number of lever presses for the same rats towards the beginning of the study and near the end. In most cases the orange bar would be taller than the turquoise bar, which shows that the total number of lever presses increased throughout the experiment. Rats like numbers two or eight in figure 3 had a higher amount of lever presses in the beginning of the study, which suggests that they experimented in between the two levers more in the beginning. What's really noteworthy about the lever presses can be demonstrated in comparing figures 1 and 2. For most of the rats, even in the control group (Rats 11-20) who received saline instead of heroin, the number of active lever presses on the last day is almost always taller. This means that the rats pressed the active lever more than the inactive lever, and that the number of times they pressed the active lever increased from the first to the last days of the experiment. This suggests that perhaps the rats enjoyed the sensation of an injection, despite the un-addictive quality of a substance such as saline.

There are also significant results demonstrated by the control group, because they were only receiving a saline solution. Some might like the way the injection of liquid felt or others might be irritated by it, and that could affect how many times they pressed the active or inactive levers. However, there is no trend between the ten rats at the beginning of the experiment and the end. This suggests that any trend found in the experimental group from beginning to end, may be worth noting.

Conclusion

Rats actively self-administer heroin which could potentially lead to addiction. It is also possible that the process leading up to the administration of an addictive drug, can be addictive. It is very difficult in an experiment such as this to determine whether a rat became addicted because it is unclear whether or not the same criterion used for humans (previously mentioned) can be used to determine the addiction of rats. However it is interesting to consider, based on those criteria, whether or not the rats could be considered addicted to the heroin. To reiterate, the three criteria for addiction are: 1) trouble limiting and/or stopping drug usage, 2) highly motivated to take drug—willing to put in work to receive the drug, and 3) continued use despite harmful outcomes (which we did not include in our experiment). Based on the progression of the rats, it became clear that over time the number of times an experimental group rat pressed the active lever increased for some rats. Rat number five went from about 15 lever presses at the beginning of the experiment to 50 lever presses towards the end. For rats in the experimental group, like number eight, whose number of active lever presses went down, that suggests that the rat may have been experimenting with the levers in the beginning and did not have as much interest in the heroin reward as a rat like number five. Rat number eight was probably not as addicted. The FR-values on the graph suggest that many of the experimental rats were willing to put in work to receive drug. At the beginning of the study, the FR-value was at one. That means that one lever push = one dose of heroin. In the later set of graphs, the FR-value was moved up to five. That means that five lever pushes = one dose of heroin. That would explain the increased number of lever pushes in the experimental group from the beginning of the experiment to the end. The rats would be confused and would continue to press levers until something would happen.

The third criterion does not apply to our experiment because no negative reinforcement of any kind of was used in this experiment. An example of a deterrent would be a shock to the foot[2]. Despite the lack of negative reinforcement, there were clear stimuli set up in order for the rats to know when they would be receiving drug (blue light, sound of syringe, etc.). Rats were never provided with a deterrent, but they were given cues that they would look for in order to know that the heroin was on its way. The process of self-administration taught the rats to associate various cues in the boxes with the reward. In this case heroin was the reward and the rats learned to act accordingly in order to receive it. These cues are set up in this way because we want the rats to learn to associate the cues with drug: to work for it and anticipate it, the way humans do. This allows taking the drug to become a habit for the rats and forces them to use parts of their brain that they wouldn't normally have to use. They are trained to remember and associate certain actions and cues with positive reinforcement.

Not all human heroin users have to be addicted just because they self-administer heroin. Just like these rats, humans can become addicted to the drug, the routine leading up to the drug, or not become addicted at all. After long periods of time though, are they more likely to become addicted because they become more reliant upon the drug. If not the drug itself, they become accustomed and possibly reliant on the routine associated with taking the drug.

This experiment demonstrates that the rats 'actively self administer heroin.' It is difficult to use a human brain disease term such as 'drug dependence'[8] or 'drug addiction' on an animal model. This is because it remains very unclear as to whether an animal really is "dependent" upon the drug. We can accurately conclude at this time that they can self-administer, similarly to humans. The rats have to decide to press the lever just as humans have to decide to inject the heroin into themselves. In this manner, this experiment shows that the different cues associated with receiving heroin as well as the ability to self-administer the drug, prompt rats (and people) to continue taking the drug. It difficult to not only stop the drug due to the effects it would have on ones' body, but also to stop the routine leading up to the administration of the drug.

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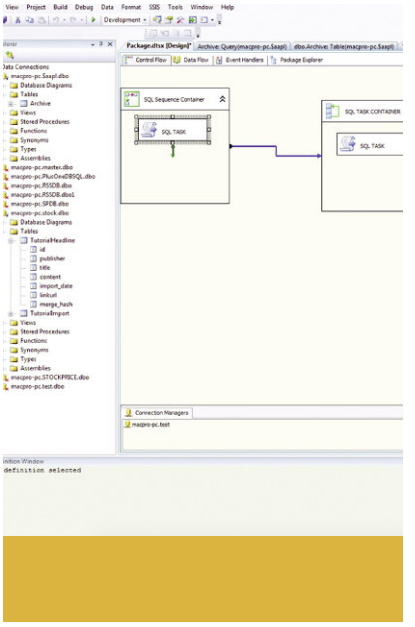
Stem Cells and 3D Cultures: The Alzheimer's Disease Research Field Today

by Julia Pike

Introduction

For thousands of years before Alzheimer's disease was discovered and named, human beings thought the natural process of aging included severe loss of memory. Forgetting was an accepted part of growing old, and hardly anyone thought it necessary to study or work on this problem (Shenk). This acceptance of forgetting was everywhere—the character of the forgetful old man even shows up in Shakespeare's King Lear (Shenk). It was not until November 25th, 1901, when a woman known as Frau D. paid a visit to Dr. Alois Alzheimer at the Frankfurt Hospital for the Mentally Ill and Epileptics, that this acceptance was challenged (Shenk). Frau D.'s case mystified Dr. Alzheimer, because she seemed to be displaying all the symptoms of an elderly person caught in the memory fog of old age—she forgot her name, her husband's name, couldn't remember how long she'd been in the hospital when asked—but she was only fifty one years old (Shenk).

Upon Frau D.'s death, five years after she'd been admitted to the psychiatric hospital, Dr. Alzheimer began to study her central nervous system, and, after years of study, he identified what we know today as Alzheimer's disease (Shenk). But what exactly *is* this devastating disease? How does it work biologically, and what changes in the brain occur when a person has Alzheimer's?

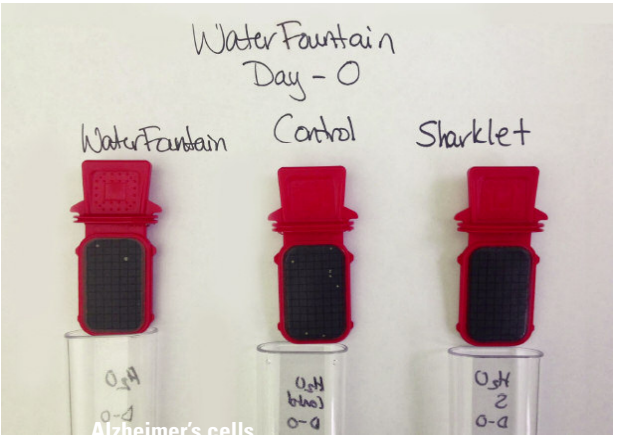


Dr. Alois Alzheimer

Background

In this paper, I will explain the current climate in the field of Alzheimer's disease research, but first I must explain the basics of Alzheimer's. The average human brain is made up of about 100 billion neurons (Alz.org). Each individual neuron is connected to several others and together, this network of cells is responsible for everything from learning new languages to writing to remembering. Alzheimer's targets these cells, the very ones that make us who we are.

The disease has two main forms of attack in its destruction of neurons: plaques and tangles. Plaques are "deposits of a protein fragment called beta-amyloid" which bundle in between neurons (Alz.org). Beta-amyloid is formed when a normal protein, amyloid precursor (APP), which is found all over the body, is broken apart incorrectly by secretase (gamma secretase), an enzyme which cleaves proteins in the brain. When this occurs, "shards of beta-amyloid" are formed, and they "stick to each other and attract detritus," which collects and forms plaques (Shenk). These plaques take up space in the brain, smashing into neurons, inhibiting their communication, and eventually destroying them altogether. Researchers believe that these plaques are the primary reason for Alzheimer's disease.



A healthy brain vs. a brain with Alzheimer's (where plaques in between cells as well as cells shrunken by tangles can be seen).

Tangles, the second destructive structure, are knotted, twisting fibers of tau protein, which is the protein in the brain that is responsible for moving the nutrients that feed neurons. Tau protein acts inside the cell itself, and therefore when the process which forms tangles through the addition of too many molecules of phosphorous takes place, tangles are found *inside* neurons themselves. These tangles stop the tau from being able to perform its function of moving nutrients through the cell, so the cell can get no nourishment, and begins to shrink away to nothing. When enough plaques and tangles build up, they cause total neural degeneration.

There are three forms of Alzheimer's disease: early-onset, late-onset, and familial. Scientists aren't entirely sure what causes early- and late-onset Alzheimer's, but from what we know it seems most likely to be a combination of environmental factors, life choices, and possibly genetic factors. Familial Alzheimer's, on the other hand, is caused by mutations in certain genes (one major gene is *PSEN1*).

Today, in 2015, it is estimated that 5.2 million Americans have Alzheimer's disease, 5 million of whom are people over the age of 65. The other 200,000 Americans with Alzheimer's are below the age of 65, and have what is called early-onset Alzheimer's. The disease affects women more than men, for reasons that science has not yet been able to determine. Of the 5 million people in the U.S. over 65 who have Alzheimer's, 3.2 million are women and only 1.8

million are men. The disease is the 6th leading cause of death for Americans and the 5th leading cause of death for Americans over the age of 65 (Alz.org).

It is estimated that if science does not find a cure for this devastating disease by the year 2050, up to 16 million people in the US alone will be affected by the disease. (Alz.org)

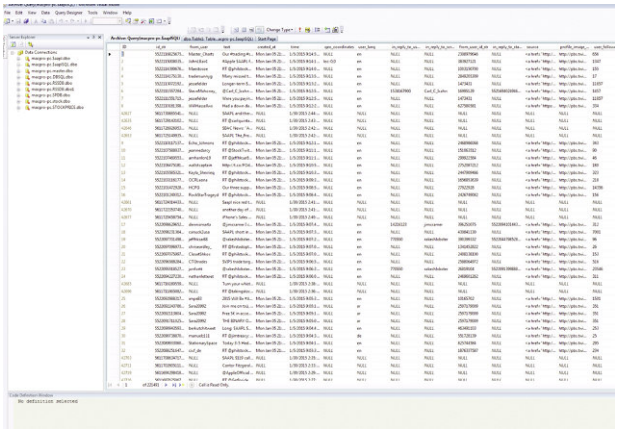
In this paper, I will explore three articles: "Characterization and Molecular Profiling of *PSEN1* Familial Alzheimer's Disease iPSC-Derived Neural Progenitors," which details the creation of stem cells with mutations in *PSEN1,* a part of the brain which deals with the cleavage of amyloid protein; "Generation of iPSC lines from archived non-cryoprotected biobanked dura mater," which explores the possibility of generating stem cells from cells stored in a biobank that were not originally intended to grow living cells; and "A three-dimensional human neural cell culture model of Alzheimer's disease," a breakthrough study which details the creation of the first disease model which contains both plaques and tangles. I will also address medicine and treatments that are used to slow the progression of the disease. My hope is that by the time you have finished reading this paper, you will have an understanding of some of the challenges and opportunities experienced by scientists working in the Alzheimer's field.

Prevalence of Alzheimer's Disease
(By Decades in USA from 1900-2050)

This graph portrays how many Americans over the age of 65 are currently affected by Alzheimer's, and a projection of how many more will become affected with it as time passes.

Characterization and Molecular Profiling of *PSEN1* Familial Alzheimer's Disease iPSC-Derived Neural Progenitors

In January 2014, I was lucky enough to have the opportunity to intern with the Alzheimer's department of the New York Stem Cell Foundation. The team is focused on finding cures for Alzheimer's disease using stem cell treatments. Stem cells are unspecialized, meaning they can differentiate into many different types of cells (blood cells, neurons, bone marrow cells, etc.). Therefore, these cells have the potential to become many different cells in the body and to aid the body in fighting many types of diseases like Alzheimer's.



Stem cells photographed using a scanning electron microscope.

There are two types of stem cells: embryonic or adult. Embryonic stem cells are derived from cells of early-stage embryos (and are thus very controversial). Adult stem cells are cells from adult humans taken (often through skin biopsies) and reprogrammed into stem cells. There are advantages and disadvantages to each type, but the Alzheimer's team of the New York Stem Cell Foundation mostly uses adult somatic cells (skin cells) through a partnership with a dermatological clinic, as these cells are much easier to acquire.

Recently, I interviewed the head of the Alzheimer's Team, Andrew Sproul, and he shed light on the team's focus and the general direction Alzheimer's research is taking. "When people think of stem cells, they generally think of regenerative medicine," meaning the use of stem cells to grow new body parts, but Sproul explained that the approach "is unlikely to work easily in the short-term with Alzheimer's because problems are all over the brain, not localized in one area where you can fix cells." He explained the current focus of the Alzheimer's team at NYCF, "is on disease modeling, [on] understanding the disease and providing a platform for drug testing."

This is good background to know in order to fully understand why the team studied what they did in the experiment we are discussing in this section. As I stated before, the purpose of this experiment was to study the effects of *PSEN1* (a gene which codes for presenilin, a protein responsible for cutting the amyloid-beta protein) through stem cells. The abstract of the article explains that "*PSEN1* mutations are the most common cause of early onset familial Alzheimer's disease," (Sproul, Jacob). The article sought to examine the specific effects of *PSEN1* by generating stem cells from "affected and unaffected individuals from two families carrying *PSEN1* mutations," and a control (Sproul, Jacob). They then examined the fibroblasts (connective tissue cells) and neural progenitor cells (NPCs, a type of very specific cell, similar to a stem cell, which differentiates into, or becomes, a brain cell) to see if either contained elevated levels of Aβ42 or Aβ40, two cells which have been shown to cause Alzheimer's plaques.

Andrew Sproul, one of the lead scientists, extended that: "What we're trying to do [is create a] disease model using patient-specific stem cells for people who have either early-onset or late-onset sporadic disease, as well as unaffected patients. Then we're trying to look for differences between them that help us understand the disease better, or at minimum provide a better platform for therapeutics."

The researchers generated stem cells and when they examined the resulting fibroblasts and NPCs, they found an increase in the amounts of Aβ42 and Aβ40 (cells that cause Alzheimer's). Additionally, the researchers examined three other genes whose expression was affected by the mutated *PSEN1* gene. They found that *NLRP2,* which is "a component of inflammasome, a protein complex that activates pro-inflammatory caspases," is upregulated, meaning there's more of it. This hints that the way the bodies of people with *PSEN1* mutations handle inflammation is different from people without those mutations, which could have detrimental affects on the brains of people with *PSEN1* mutations (Sproul, Jacob). Researchers found that ASB9, a gene which, when overexpressed, has negative effects on the mitochondria (the powerhouse of the cell), was upregulated as well. Lastly, researchers found that there was an upregulation in NDP (a gene which plays a part in Norrie disease, which causes blindness, hearing loss, and can cause mental retardation). This led researchers to question whether familial Alzheimer's disease "has a developmental component," (Sproul, Jacob).

This was an incredibly interesting experiment with two main results. It worked to further prove that *PSEN1* plays a part in the creation of more Aβ42 and Aβ40, so essentially that it leads to familial Alzheimer's disease. It also provided more pathways for study by examining the different genes that were upregulated by the *PSEN1* mutation.

Generation of iPSC Lines from Archived Non-Cryoprotected Biobanked Dura Mater

At the same time, the NYSCF Alzheimer's team was working on a different experiment in a slightly different vein. In my interview with Andrew Sproul, he explained how much of an obstacle uncertainty in diagnoses can be. "It's difficult to know for sure if someone has Alzheimer's unless they're dead. Right now, testing (using biomarkers and brain imaging) is at a 97% accuracy when patients are alive," although that seems like a good percentage, the 3% uncertainty can cause huge problems for researchers. Deceased patients' brains, on the other hand, can be more definitively diagnosed, because they can be examined more invasively post-mortem.

This leads to the reasoning behind the second experiment. Researchers wanted to test whether stem cells could be grown from brain and scalp cells of patients who had already died. This would erase the question of uncertainty of diagnosis. The only problem was that the tissue used had not been originally intended to be used to grow live cells. It had not been cryoprotected (basically, protected by freezing agents), in order to ensure that every component of the cells that was needed in order to reprogram them into stem cells would remain intact.

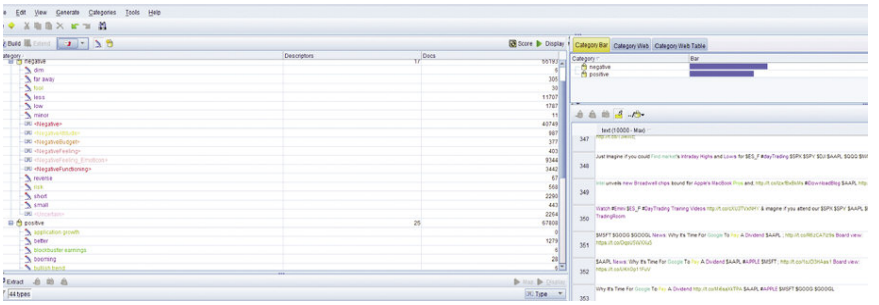
In order to test whether these cells were, in fact, usable, scientists used tissue which had been stored at -80° for as long as 11 years. Cells from the brain were reprogrammed using a virus, which infected the cells and induced them to revert back into stem cells.

The results of the study were clear-cut: "both scalp and dural cells can be reprogrammed to produce high-quality" stem cells (Sproul, Vensand). The one drawback was that the yield of the stem cells produced by the non-cryoprotected cells was slightly lower, but researchers did not seem to think this was a significant issue. Researchers highlighted how important this finding was, noting that there is a "vital need for well-characterized patient material for translational research," (Sproul, Vensand). These findings can help assuage some of the uncertainty that currently plagues this field.

A Three-Dimensional Human Neural Cell Culture Model of Alzheimer's Disease

"A lack of a viable model for Alzheimer's has been the Achilles heel of the field," explained Dr. Doraiswamy, a researcher at Duke University (Kolata). But in a breakthrough study, this issue has been somewhat remedied. Researchers Rudolph E. Tanzi and Doo Yeon Kim have found a way to grow an Alzheimer's model in a dish that formed both plaques and tangles, something that models haven't been able to do thus far. "We have successfully recapitulated amyloid-β and tau pathology in a single 3D human neural cell culture system," the researchers' paper declares (Tanzi, Kim).

How exactly what this done? Researchers "overexpressed human β-amyloid precursor protein [the protein whose incorrect cleavage causes the sticky amyloid shards which result in plaques]... and presenilin 1 *(PSEN1)"* in stem cells (Tanzi, Kim). This had been done before, but the novel approach these researchers took was to culture the cells in 3D.



Nerve cells (in green) showed surrounding the artificially grown Alzheimer's plaques (in orange).

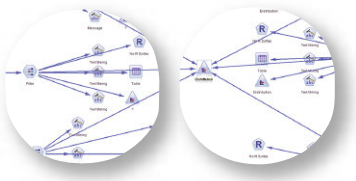
"In conventional 2D cultures," the article explains, "secreted amyloid beta diffuses into a large volume of media." Essentially, the cells are swimming in large amounts of media, and so forming amyloid structures in all that media can be difficult. But a 3D culture "accelerate[s] amyloid-β deposition by limiting diffusion of amyloid-β, allowing for aggregation," (Tanzi, Kim). Essentially, when the cells had the 3D model to grow on, amyloid-β and tau were able to form much more easily.

With the creation of the 3D model, tau structures were able to grow, which "shared a striking similarity with those observed in the brains of Alzheimer's disease patients." This was the most important distinction; with other models, tau had been detected, but never in the tell-tale tangle structures of Alzheimer's disease. At the end of the paper, the researchers explain that they have "successfully recapitulated amyloid-β and tau pathologies in a 3D human neural cell culture system, which can be used as a platform for studying the pathogenic mechanisms of Alzheimer's disease and drug screening," (Tanzi, Kim). This experiment was also extremely important because it was strong support for the amyloid hypothesis, which is the idea held by most scientists that Alzheimer's is caused by amyloid rather than tau, and that the reason tangles form is because of amyloid plaques. The fact that the overexpression of amyloid-precursor protein caused plaques and tangles to form "provide[s] experimental validation of the amyloid hypothesis of Alzheimer's disease, which proposes that the accumulation of amyloid-β drives tauopathy," (Tanzi, Kim).

Current Treatments for Alzheimer's Disease

Today, there are no drugs that reverse the progress of Alzheimer's, but there are drugs that slow the progression of the disease. The drug most often used to treat Alzheimer's is called Aricept. Aricept "works by preventing the breakdown of a chemical called acetylcholine," and inhibiting cholinesterase (Drugs.com). Acetylcholine is a chemical that is released by cholinergic neurons, which are a type of neuron that helps with sleep and alertness. These neurons are one of the first to be attacked by Alzheimer's disease. Cholinesterase is an enzyme that controls the activation of cholinergic neurons and breaks down acetylcholine. Essentially, the Aricept's job is to increase the amount of acetylcholine in the brain so that cholinergic neurons that have not yet been destroyed can work more effectively, and decrease the amount of cholinesterase so these few neurons aren't so heavily regulated and acetylcholine isn't broken down.

In the past, it was believed that the imbalance in cholinesterase and acetylcholine was a big factor in Alzheimer's disease, but today researchers think that it is only a small part in a larger network of issues that Alzheimer's patients' brains undergo. Still, large amounts of research into treatments related to this and other chemical imbalances are being done today, in efforts to learn how best we can combat this devastating disease.



The other major treatment aside from medicine is therapy. Although therapy is unable to slow or stop the progression of the disease, it can be essential in helping patients to come to terms with the disease. Group therapy is often suggested for patients with Alzheimer's, as being in a controlled setting with others who are experiencing the same decline can be comforting; Alzheimer's patients often feel very alone as they progress further into the disease, but individual and group therapies can be incredibly helpful in combatting these feelings.

Conclusion

Alzheimer's disease has wide-reaching and devastating effects. In this paper, I discussed many of the things that are being done to take steps toward eradicating Alzheimer's: studies on brains' of already-deceased patients, experiments dealing with the creation of neurons through stem cells, modeling of the disease through 3D cell cultures. Each of these things alone may seem small and futile when held up to the terrifying force that is Alzheimer's disease, but it's important to remember that this type of science is like putting together a puzzle. One study reveals that non-cryoprotected brains can be used to generate stem cells, and a puzzle piece falls into place. Another study creates viable model of the disease, another piece is put into the puzzle. It's slow going, but it's essential to remember that this process of micro to macro is the essence of science itself. It's how scientists change the world.

In this field especially, though, science has a long way to go. Many questions still plague the field, and hint at future research that must be conducted. What causes early-onset Alzheimer's? What environmental factors cause the non-genetic form of Alzheimer's? Is a 3D culture the most effective way to model Alzheimer's, or are there better models we have yet to discover? Is the amyloid hypothesis correct or, in other words, is Alzheimer's caused by the accumulation of amyloid plaques? Or do tangles play a larger factor than scientists now think? These are just some of the many pressing questions that need to be researched if we are to combat this disease.

Human Impact

One of the many issues with this disease is that in the scientific study of the particulars, the human impact can often get lost. I had the opportunity to interview Mr. X, who will remain anonymous, and learn more about this impact. Mr. X has been helping care for his grandmother, who was diagnosed with Alzheimer's ten years ago at the age of seventy-four. In that time, he's watched her decline steadily. "She's reached the point where she doesn't know anyone outside of the immediate family. She doesn't know her cousins, she doesn't know her sisters, people who come over to the house that she hasn't seen in maybe a couple months—she doesn't recognize them, she doesn't know who they are. She'll say hello to me three or four times within the first ten minutes that I'm at the house, and she'll say goodbye to me three or four times within the last five minutes that I'm in the house." In terms of care, he explains that his grandmother "can still feed herself, she can bathe, but she needs to be reminded to do so," he explains. "If we didn't tell her to bathe, then she would never bathe, and if we didn't prepare food or tell her that it was lunchtime, she wouldn't eat." When discussing the medication his grandmother was prescribed at her diagnosis "to manage and slow down the Alzheimer's process itself," he explains that he "hasn't seen it make a difference."

Mr. X describes the process of caring for his grandmother as a complicated one, because she's so far into the disease that it's hard for her caretakers to do anything to help her. "There really isn't a caretaking process to her. We actually wind up taking care of my grandfather, since he's been watching over her," he says. In David Shenk's book *The Forgetting,* he describes a condition known as "caregiver's dementia," which is the "fatigue and forgetfulness" that comes along with taking care of someone who has Alzheimer's (Shenk). Mr. X described these symptoms in his grandfather: "We've seen, over the years, my grandfather's health decline while taking care of my grandmother: his overall demeanor, his body weight, the actual physical look in his face. Now that we've moved them into my parents place, and now that he's been there and not alone with just my grandmother, we've seen his health come back. He's gained weight. [Before], it was almost like, a sunken face, a beaten, stressed look. But I've definitely seen more of an effect on him than on any other member of the family. Caring for her over the past ten years I think has really worn him down a lot."

In my interview with Mr. X, he told a story that seems to trouble him. He explained that his grandmother has been a smoker since she was thirteen, so for more than seventy years. "She continues to smoke, and she'll forget that she just had a cigarette so she'll go back out, so she'll go through three packs a day," he explains. "My dad, in his futile attempts, has told her to leave the cigarettes outside, but she'll leave them in the garbage [indoors], and so the whole house starts to reek like cigarettes. Every time she comes back in and she puts them in the garbage. I don't know why she does it. My dad sometimes will raise his voice at her and tell her that he's told her multiple times, that he doesn't want her putting them in there, but I've noticed that she will actually sneak them back in the house and put them in the garbage, which is interesting. We have things outside for her to put the cigarettes in. What compels her to bring them inside the house? And to hide them in the garbage?" This particular story stuck with me, as a reminder of the confusion Alzheimer's causes—among those who suffer from it, among family members of those who have the disease, among scientists. It is the essence of why research in the Alzheimer's field can be difficult and frustrating, but it's also another reminder of how necessary the work truly is. It proves that although scientists may feel caught in the micro of the work, the effect that a macro development could have would change millions—perhaps billions—of lives in the coming decades.

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Images

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Tints & Tones: The Hidden Connections Between Color and Sound

by Sophie Hayssen

Abstract

This experiment addresses how our cross-sensory perception of color and sound changes with age. This question was tested by having participants match colored cards with musical notes varying in pitch. The data showed a positive correlation between age and the tendency to pair dark colors with low tones. The consistency of the pairings also increased with age. This consistent, interconnected sensory perception is also seen in people with the neurological phenomenon synesthesia, but at a much more extreme level. Therefore from this study, we can conclude that regular sensory perception becomes more similar to synesthesia after a certain age.

Background

This study looks at how sensory perception in human beings develops over time. In particular it examines the brain's ability to make connections between two or more sensory modalities, other wise known as cross modal perception (CMP). CMP is believed to account for our understanding of metaphor since the nature of these connections is abstract (Harvey). Scientists provide concrete examples of CMP in experiments like the one conducted by scientists Lawrence Marks and Gail Martino, which show that people were faster at classifying high pitched tones while looking at a white square and low pitched tones while looking at a black square. This demonstrates the existence of CMP because the color of the square affected the time it took to classify the pitch. In other words, it showed that humans inherently associate colors and pitches (Marks & Martino).

Cross modal perception fits into the larger topic of synesthesia, which is a condition where two sensory modalities can be elicited by the same stimulus. Scientists believe that the two are in some ways linked. Some even refer to CMP as weak synesthesia. People believe this because it has been proven that synesthesia and CMP have inherent similarities (Harvey). The study conducted by scientist Jamie Ward corroborated the results of the Marks and Martino experiment by comparing the pairings of particular colors and sounds for sound color synesthetes to those of non synesthetes. The data showed that both groups followed a similar trend; they tended to associate lighter colors with higher pitches (Ward).

This is not the only connection between the lightness-pitch association and synesthesia. A lot of scientists believe that CMP is evidence of the neonatal synesthesia hypothesis, which argues that we were all born with synesthesia but due to genetic pruning those connections are cut off when we are young (Maurer). CMP is the result of incomplete pruning, connections that were mitigated but not entirely destroyed. CMP's connection to the neonatal hypothesis is supported by the fact that this association is said to be innate, a connection people make that requires no prior teaching or experience (Maurer). This was discovered when scientists tested

young children (30-36 months) and found that while watching a movie depicting "two balls bouncing in synchrony with each other and an overall sound that varied in frequency," the children consistently identified the lighter colored ball as making the lighter sound (Maurer).

This experiment aims to question the conclusions of Ward's experiment, examine the relationship between CMP and synesthesia, and reconsider the neonatal hypothesis. For example, if the data showed a correlation between pitch and color lightness it would provide further support for Ward's claims. It would also support the theory that CMP and synesthesia are somehow related. Depending on how the correlation and consistency changes with age, these findings might also call the neonatal hypothesis into question.

Materials and Methods

The first step in designing the experiment was to assemble the appropriate series of musical tones and colors that would be used to create the test. The colors were found by comparing swatches of grey paint, and selecting five from the stack that represented a very clear range of shades. This range consisted of one very light shade, one light shade, one medium shade, one dark shade, and one very dark shade. These paint swatches were then scanned onto a computer and used as choices for the test.

The other important player in the experiment was the tones that were recorded on the built-in keyboard of iTunes' GarageBand. There were five tones in total, which like the colors were assigned pitch values from one to five, five being the lowest note. By attaching these numerical values to both the colors and the pitches I could derive quantifiable results from my data.

This study tested 24 subjects in total. The pool was made up of 9 kindergarteners, 11 juniors, and 4 adults. All participants were selected from the population of the Berkeley Carroll School. The kindergarteners and juniors tested all came from the same class and were all tested at the same time. The adults, all members of the faculty, had to be tested individually at different times due to scheduling. All adults were in their 30's or 40's.

In this experiment the tests administered to the participants were created using a online survey creator called Survey Monkey. The tests were composed of 5 questions each with every question showing some random arrangement of the five shades. Each shade was labeled option 1-5 depending on its position in the question (see appendix).

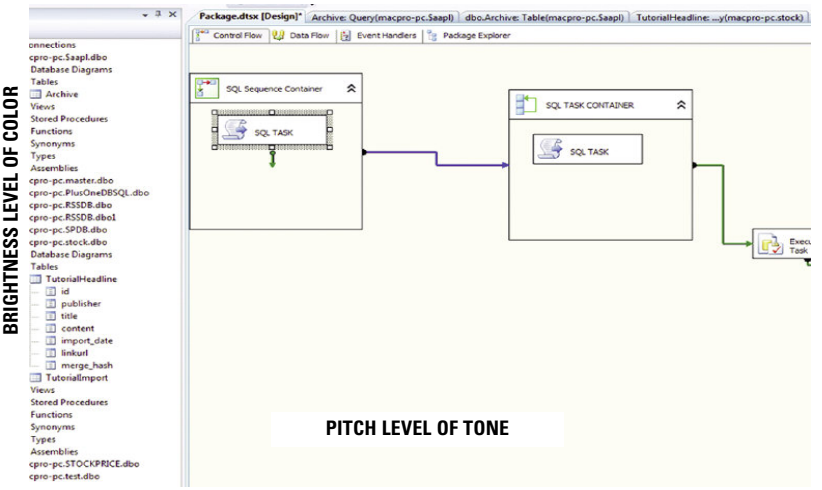
When notes were played the participant would click the color that they thought best fit the note. In order to keep track of which option corresponded to which number I attached lightness values to each of the shades with 1 being value for the lightest color and 5 being the value for the darkest color. In order to keep track of which color corresponded to which option, I made a key for each of the questions. The key was known only to me and my advisor and used solely for analyzing data. As with the order of the colors, the order of the tones was also random. One tone was played per question with each tone played five times in a row to ensure that the participants heard it clearly and give them time to make their selection.

Each participant was tested three times. The first two tests occurred on the same day and the second, about a week to two weeks later. This controlled for the outside variable, memory. For two of the three groups tested, the participants recorded their answers directly on the online survey. However for the third group—the kindergarteners—that did not have access to technology, they were merely shown the different options on an iPad and then recorded their answers on paper.

Results

Figure 1

Correlation between Lightness Levels and Pitch of Tones for Kindergarten



R value (degree of correlation) = .38, therefore there is an insignificant 38% positive correlation between color brightness and pitch level. The darker the diamond, the more subjects made that selection (more subjects chose a pitch level of 3 with a brightness of 3 than with a brightness of 4)

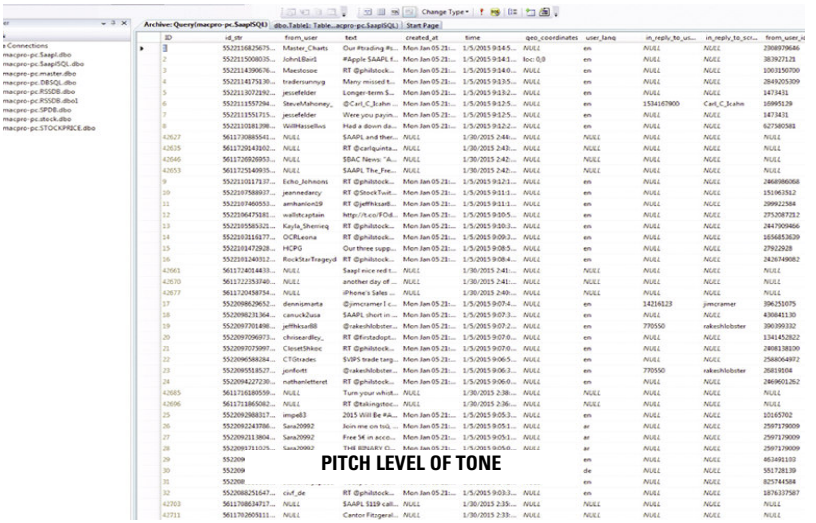
Figure 2

Correlation between Lightness Levels and Pitch of Tones for Eleventh Grade

The R value (degree of correlation)= .76 meaning that there is a significant 76% positive correlation between brightness level and pitch for eleventh graders.

Figure 3

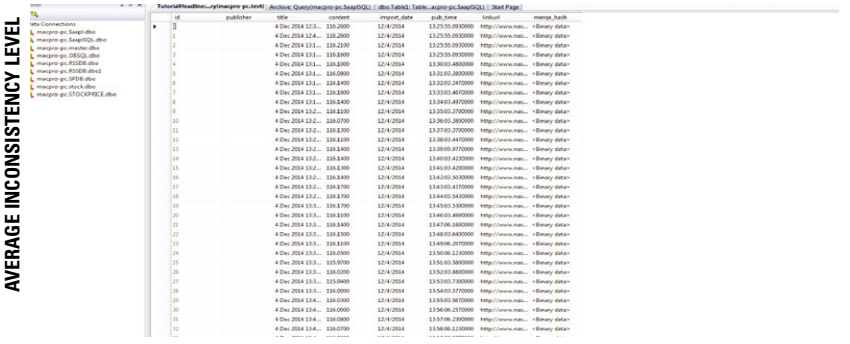
Correlation between Lightness Levels and Pitch of Tones for Adults



R value (degree of correlation) = .96, meaning there is a significant 96% positive correlation between color brightness and pitch level for adults

Figure 4

Average Inconsistency Across Age Groups



ANOVA test yielded a p-value of .0001, meaning the differences between the amounts of inconsistencies is significant. Note kind stands for kindergarten. Inconsistency values were defined as the summation of the differences between color choices (assigned a numerical value from 1 to 5) for all five tones across the three tests.

Discussion/Conclusion

Two prime conclusions can be drawn from the data. The first is that the correlation between color brightness and pitch becomes increasingly significant with age. As we can see in Figures 1-3, the connection between these two variables begins at an insignificant 37% during the

kindergarten years. By eleventh grade that number doubles and reaches a significant 76% correlation. This only increases in the adult years when the number reaches 95%. What we can gleam from this is a partial backing of Ward's observations with the addendum that there is only a connection brightness and pitch in non-synesthetes after a certain age. This turning point appears to fall somewhere in the 10 years between ages 6 and 16.

The second conclusion one can draw from the data is that people are more consistent with their pairings as they get older. This is shown in Fig 4. The ANOVA test conducted on the three sets of data delivered a p-value of less than .0001, which indicates that there was a significant difference in the consistency levels across the three age groups. Again, as in the case of the first conclusion, there was a significant drop in the inconsistency level between the kindergarteners and juniors.

Because CMP took on traits found in genuine synesthesia, these findings imply that there is a connection between them. What this study doesn't show is how deep this connection runs or where it begins; is the connection biological or environmental? It is also noteworthy that CMP only became similar to synesthesia for older participants (juniors and adults), which opens up the possibility that CMP might be learned as opposed to inherent. If this is the case it would challenge Maurer's evidence for the neonatal hypothesis. The idea that CMP is learned instead of innate would indicate an absence of residual neural pathways from before pruning took place. On the other hand, if we supposed the neonatal hypothesis was true these findings would give us a timeline. By age five or six our brain would have formed in such a way that any residual neural pathways would not function in ways that resembled synesthesia. As the results show, there is no correlation or consistency for young children. But then as we got older these neural pathways would grow stronger, our associations would become fixed, and our ability to conduct CMP would increase.

There is another explanation as to why this transformation occurs, one that could counter the neonatal explanation. It may not be a coincidence that such a great change in the strength and consistency of the participant's associations occurred in the same particular age gap (6-16), which just so happens to be the time where the brain undergoes drastic changes during puberty. One book called The Adolescent Brain: Learning, Reasoning and Decision Making cites several scientists who developed a theory that as humans develop they move from "concrete thought to more abstract understanding" (Reyna, 97). The reasoning of young children they say are "more tied to perceivable properties" (Reyna, 97). These ideas corroborate the data. Though they don't explicitly talk about sensory perception it can be inferred that the same kind of development would occur on that plane of thinking as well. For kids, it seems the world may appear to be one great ball of information. The adult and even adolescent brain may have developed to be more adept at processing this information and analyzing it at higher levels of thought. It might be worthwhile to consider whether CMP develops as a result of abstract thinking. If this abstract thought evolved independently of any residual neural pathways then it could challenge the neonatal hypothesis.

There were several sources of error in this experiment. Due to scheduling conflicts and time constraints the time between administering the 2nd and 3rd tests were differed between participants. This could have affected the data because it makes the data slightly vulnerable to memory. Perhaps the people for whom the elapsed time was shorter could have better recalled their own connections. A second possible source of error occurred with the kindergarten class who didn't have iPads of their own and had to cluster around mine to see each question. This

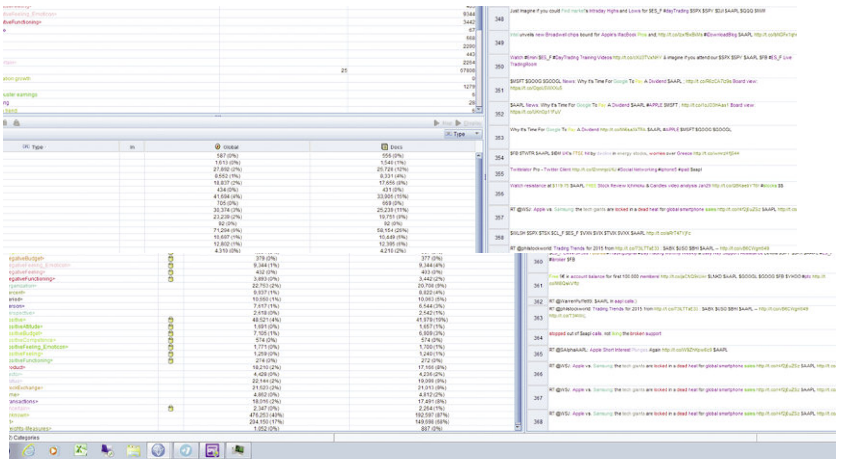
set-up might not have given them enough time to look at the question and really see each color. Because they all had to cluster around one iPad they were in very close proximity to one another which opens up the possibility that they might have accidently seen each other's papers. In addition, kids would sometimes call out their answers after hearing a tone that likely swayed or affected some of their results. One of the adults tested also told me beforehand that she was a synesthete. While she had never been officially tested she told me that she sees days of the week as particular colors. This technically shouldn't have mattered for the purposes of the experiment because she was not a sound-color synesthete, however there is a possibility that it did affect her answers. Lastly, I only tested four adults. Granted, all their answers followed the same trendline. However, it is still a very small amount of people to base a conclusion off of. If I had more time, I would have liked to test more adults to lessen the chance of error.

The study and its results raised several questions that, if answered, might shed new light on these results. One of these possible directions for future research is whether or not the medium on which the study is conducted would affect the results. This answer might conclude whether the kindergartener's use of paper instead of an iPad to complete the tests in any way affected or skewed their results. Second, and perhaps more important, is the case of the one adult participant who identified as a synesthete though not a sound-color synesthete. A possible experiment would be to see if a synesthetes' pairings between other sensory modes are affected, even if it does not relate to his or her specific kind of synesthesia. Perhaps one might even explore if the relationship between different sensory modalities are affected in different ways for example, perhaps a sound color synesthete may have very fixed, synesthesia-like pairings between numbers and colors, because that kind of synesthesia might use similar areas of the brain but have little to no rigidity in their lexical-gustatory pairings. Lastly, since this experiment only tested some adults and no senior citizens, it might be worthwhile to see if there is a point where our ability to compartmentalize and sort these different senses peaks at a certain point.

Appendix

SRD Experiment Part 2

Note 2



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We Need to Be More Positive: The Psychology of Procrastination

by Lucy Beers Shenk

Introduction/Literature Review

Procrastination affects all of us. In one way or another, each person on this Earth has delayed a project or obligation. However, there are people who procrastinate twice a year, and there are people who procrastinate twenty times a day. For some, it can become a more serious problem, almost an addiction. I have struggled with procrastination for a number of years, so last January I decided to look into it. What I found was surprising and fascinating. While I, along with the rest of the world, always thought of procrastination as just someone being "lazy" and not being a "hard worker", psychologists are now coming to the conclusion that it is a psychological problem.

Procrastination mostly affects people who are perfectionists. They are afraid of failing, and subconsciously tell themselves a task will be too hard for them, so they can't bring themselves to begin the project at hand. The hard work will be so difficult that they tell themselves they will suffer no matter what, so why not suffer for a short period of time instead of a long period of time? A procrastinator will tell her/himself that they can be happy *now,* relax *now,* and that is much more appealing than working on something difficult and, in their eyes, almost impossible, *now.* Procrastinators make trade-offs. They choose short-time gains (watching television now instead of working on an essay), and push away the thought that they'll be forced to experience long-time failures (fall behind on the essay, struggle with the stress of last minute editing, lose sleep, possibly get a worse grade than they would have if they didn't procrastinate). It becomes a cycle of negativity that is extremely hard to break out of (Tice, "Student Academic Services").

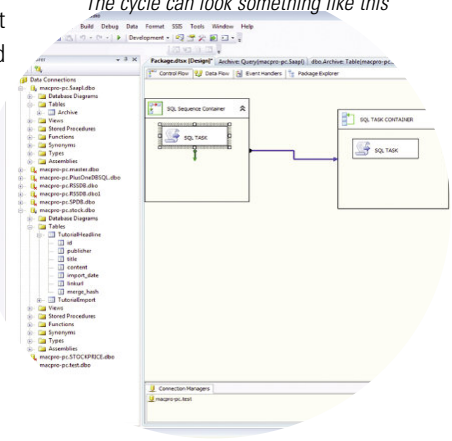
I have read a number of studies on procrastination. One study followed a class over the course of a semester. The class had a paper due at the end of the term. The results showed that the students who put off the paper until the last minute, and who procrastinated more over the course of the semester, were more prone to becoming sick (probably due to lack of sleep after they couldn't manage their time well), and they generally got lower grades in the long-term than those who procrastinated less (Tice). Again, short-term gains, and long-term failures.

Today, around 20% of the United States are chronic procrastinators ("The Organization Against Chronic Procrastination"). People struggle internally with procrastination every day, trying desperately to keep it from taking over their lives. With all of the technology and opportunities available to us, we are always being surrounded by so many distractions. Recently, I have been having more

Figure 1

The Procrastination Cycle

The cycle can look something like this



conversations with more people within the Berkeley Carroll community who experience serious problems with procrastination, which has made me wonder, are we the procrastination generation? Are we in an era of procrastination? How many people in our school are struggling with the same things I am struggling with?

Pilot Study

Last spring, I conducted a pilot study at the Berkeley Carroll School in which I used a one-time questionnaire that I designed to see if there was a correlation between procrastination and negative well-being. Do people who procrastinate more than others feel worse about themselves? I hypothesized that there would be a correlation; that as procrastination increases, negative well-being would increase as well. I also was careful to not use the word "procrastination" in the survey and when informing the participants of the survey. I told them it was about "work habits". The survey consisted of a set of "procrastination" questions, and "self-perception" questions (the questions were mixed up and in no particular order). The participants answered the questions on a scale of 1 to 10, where certain points on the scale were labeled (for some questions, the labels on the scale had to be varied from how they appear in figure 2).

Procrastination Questions:

Do you ever put off a task?
Do you have trouble making decisions?
Do you ever get "choice paralysis" — when you can't make up your mind?
Do you believe (either subconsciously or consciously) that you can only be happy when you're relaxing ("all pleasure comes from leisure")?
Do you tell people/tell yourself that you do your best work under pressure?

Self-perception Questions:

Are you afraid of failing?
Do you wish you were more productive?
How often do you tell yourself that you are lazy?
How do you feel if someone tells you to do something that you haven't yet done?
Do you feel good about yourself?

Scale points for self-perception questions

1: I am almost always happy with myself
4: Generally happy with myself
7: Sometimes happy with myself
10: I am almost never happy with myself

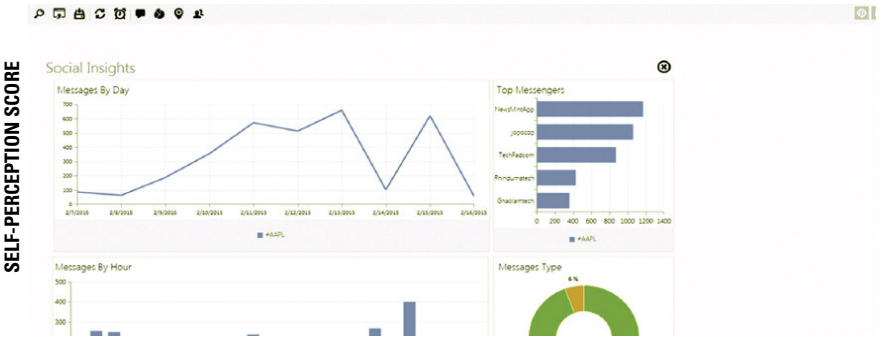
Scale points for procrastination (work habits) questions

1: Barely ever put off my work
4: Almost never put off my work
7: Generally put off my work
10: Almost always put off my work

I did a simple random sampling of the tenth graders in my high school, and sent them the survey, and reminded all of them many times to fill it out. I found that there *was* a correlation between the two factors. The graph below shows the plotted points of the total self-perception score and procrastination scores of each participant, therefore confirming the fact that people who struggle with procrastination think badly of themselves.

Figure 2

Procrastination vs Self-perception



Full Study

My next step was to examine exactly how negative self-perception feelings *happened* in the first place. I guessed that because I was following high school students who procrastinated on assignments that were graded by teachers, the negative feelings could come from the teachers—whether the teacher gave them only negative feedback on the project at hand, and how the teacher made them feel. Did the teacher make them feel, maybe because of past projects, that they couldn't succeed? Did the student therefore go into the experiment feeling discouraged and assuming from the beginning that they were bound to fail?

Another possibility is the outside stress factors from parents—do the participants' parents put a lot of pressure on the student to succeed perfectly, or do the parents tell the student that they are going to fail?

I tried to design an experiment around these parameters, and the hypotheses I was making, based on logic and what I know about people who regularly procrastinate. I carefully wrote a number of questions and specific parameters for responses. The survey looked like this:

How much progress have you made on this project?

Answer Possibility: You could answer any number 1 through 10, 1 labeled as "None", and 10 labeled as "Done".

How much positive/negative feedback have you received on this project from an outside source (teacher, parent, tutor)?

Answer Possibility: You could answer as "I haven't received any feedback", or any number 1 through 10. 1 was labeled as "I have received very negative feedback on my work", 5 was labeled as "I have received an equal amount of negative/constructive and positive feedback", and 10 was labeled as "I view the feedback I have received only as positive feedback".

How do you feel about how you will do on this project?
Answer Possibility: You could answer any number 1 through 10, 1 being labeled as "I'm going to fail, probably", and 10 being labeled as "I'm going to get an A".

How has this teacher made you feel in the past?
Answer Possibility: You could answer any number 1 through 10, 1 labeled as "Like I can't succeed at all", 10 labeled as "Like I can always succeed".

Do you feel like you should be working more on this project? What have you been doing instead? Please be specific.
Answer Possibility: This answer was just a text box open for the participant to write as much or as little as she/he wanted.

It was important that I defined some of the numbers on the scales, because then each participant was looking at the scale roughly in the same way. Otherwise, one person's "8" might not mean the same thing as another person's "8".

I sent the survey out every other day for eleven days starting on December 8 and ending on December 18.

Data

Figure 3

Progress vs Confidence

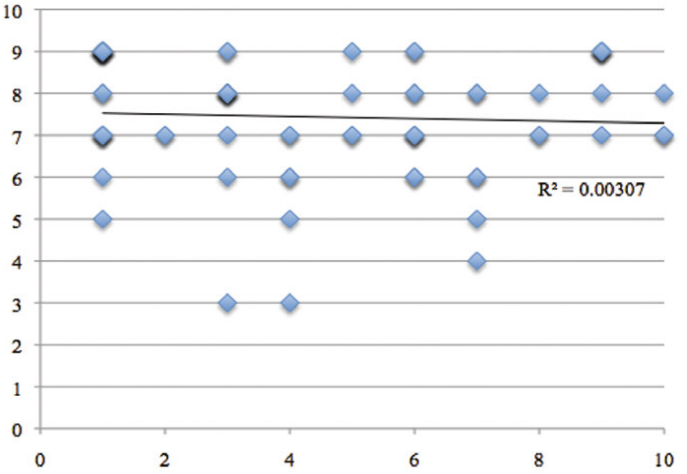


Figure 3 shows that there is no correlation between the progress the participants made on the project (whether or not they procrastinated), and how confident they felt about the project.

Figure 4

Progress vs Past Teacher Feedback

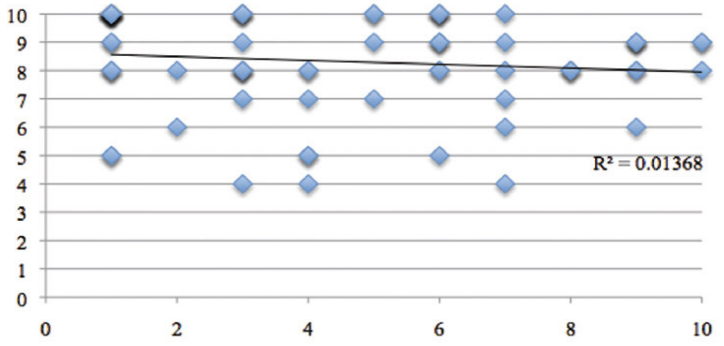


Figure 4 shows we can see that there was no significant correlation between the progress participants made, and the feedback they had received from their current teacher in the past.

Figure 5

Progress vs Positive Feedback from Teacher/Parent/Tutor

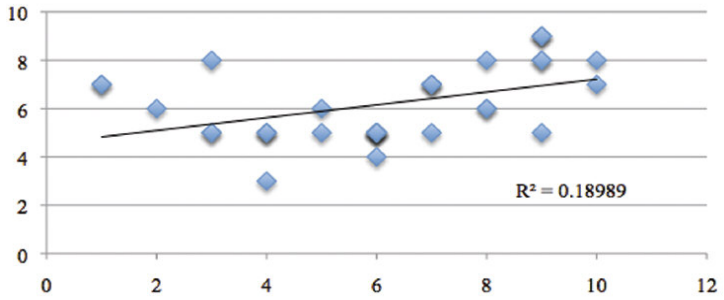
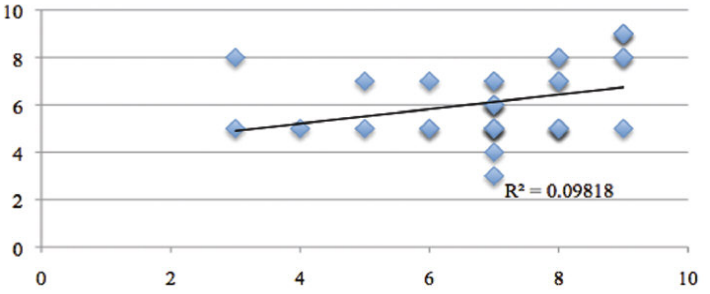


Figure 5 shows there is a slight correlation, as demonstrated by the upward curve, between the progress made over time, and the positive feedback participants received from outside sources. This is the kind of thing I was expecting in my study—for people to have heard some sort of message that they can succeed, and that makes them procrastinate less.

Figure 6

Confidence vs Positive Feedback from Teacher/Parent/Tutor



Naturally, figure 6 shows that students have more self-confidence when they receive positive messages from outside sources. We can assume, to an extent, that this could decrease the amount of procrastination.

Figure 7

Confidence vs Past Teacher Feedback

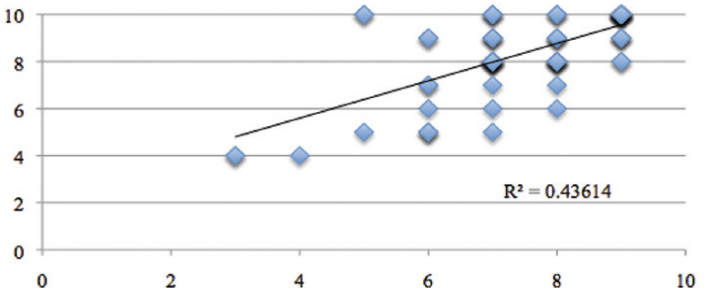


Figure 7 shows us the biggest and most significant correlation from my study. Participants had more self-confidence in the fact that they would succeed in this project, and overall, the participants with more self-confidence had received positive feedback in the past from their current teacher.

Analysis of Data and Areas of Error/Uncertainty

After reading through all of the data, I realized I had very mixed responses on how the teacher made the student feel, and how much feedback the student had received. I was confused that some of the participants' numbers representing how much progress they had made went up and down. I should have clarified that I wanted them to note how far overall they were with the project. I noticed a few other mistakes on my part as well. The students were not all in the same class, and some teachers have different effects on others. And, I did not realize that some final projects did not have two rounds of editing—so a lot of participants had to put "I haven't received any feedback".

Despite the range in answers, I found that most students put off their assignment until the last night or the last two nights. There were a couple exceptions to this, but I don't think enough to draw a bigger conclusion out of it. Most people wrote to me that they had been

focusing on work for colleges. Some people said that they knew they should be focusing on the project, but they weren't. All of this leads me to believe that, overall, the data I collected in my full study was inconclusive.

Still, the last graph, looking at confidence versus past teacher feedback, shows a fairly significant correlation. Students were more confident in their ability to succeed and do well in this project when they had received positive feedback from their teacher in the past. Although there is no data that shows that this decreased the amount the students procrastinated, it is important to note that the students felt they could succeed on this project based on their own mind and hard work—and their teacher's positive feedback.

However, from being around high school students every day, and from the reading I have done, I firmly believe that students who do not procrastinate on a regular basis get better grades, and have much more positivity in their lives—they are confident that they can succeed, and that only helps them to succeed more. Students who procrastinate more often feel badly about themselves, do not succeed, and fall into a cycle of negativity. I think that it is extremely important that students who have trouble with procrastination get help, and the biggest help we can give to start is giving positive comments on what they are doing well, and constantly reminding them that they *can* succeed. When they start to tell themselves that they can achieve their goals, they will get there. For inspiration, I'll leave you with this Marianne Williamson quote:

"Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light, not our darkness, that most frightens us. We ask ourselves, Who am I to be brilliant, gorgeous, talented, fabulous? Actually, who are you not to be?"

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Stem Cells

by Abigail Marin

Abstract

Stem cells have been emerging as one of the biggest areas in medical research of late. And there is so much to research and learn about them. We have all seen them on the news. They have been used to grow everything from new organs to a burger in labs. As many of us may already know, stem cells have the potential to treat many diseases. But there are many ethical laws limiting their use[13]. This literature review discusses the uses of different types of stem cells—specifically cord blood stem cells. In order to understand how stem cells can be used as a treatment, this literature review also includes a case study on the effects of a cord blood stem cell reinfusion as an experimental treatment on children with cerebral palsy.

Stem Cells

Cells are the smallest unit of life. There are many different types of cells in the human body and each has a specific function. But, there are some cells that are undifferentiated; meaning that this type of cell does not have a specific or specialized function.

When cells are undifferentiated they are not expressing any genes that will help them carry out a specific function. However, undifferentiated cells can become specialized cells or differentiated cells through the process of differentiation. Differentiation is when cells become a specific type of cell in a specific tissue for a specific job. This process occurs when the cell receives external signals telling it to start becoming specialized. When the undifferentiated cell receives these signals it starts to express genes for specific functions. The proteins that result from the expressing of specific genes allow the cell to perform jobs, differentiating from other cell types in order to become one type of cell. After receiving more signals the cells will continue to express more specific genes and therefore become a specialized or a differentiated cell[6].

Undifferentiated cells are also known as stem cells in a multicellular organism. They are capable of giving rise to indefinitely more cells of the same type, and to cells of a specific type as they undergo differentiation. Stem cells are pluripotent. Pluripotent cells are cells that are capable of turning into almost any cell in the body. Since they have the potential of becoming any kind of cell they also have the potential to be used for the treatment of many diseases such as some cancers, blood disorders, immune disorders, and metabolic disorders as well as autism, cerebral palsy, pediatric stroke, traumatic brain injury, and acquired hearing loss in the future[12]. These disorders are now being tested with some stem cell studies.

There are three main types of stem cells: adult stem cells, embryonic stem cells, and perinatal stem cells.

Adult Stem Cells

Adult stem cells, also known as somatic cells, are found in many tissues in the body such as brain, teeth, bone marrow, muscle and adipose, and testicles. But, they have a limited ability to give rise to various cells in the body. This is because somatic cells are pluripotent. Adult stem cells are easier to use today because there is less of an ethical issue of using them since they are from already developed organisms. The main ethical issue with adult stem cells is that using the cells to grow a new organ or tissues can be considered cloning. Cloning is the copying of an organism with 100% of the same DNA[13].

Embryonic Stem Cells

Embryonic stem cells are from human embryos that are five days old. They are harvested from a blastocyst by scooping them out.[14] The harvesting of the stem cells from a blastocyst is pictured in figure 2.

Figure 1

Adult Stem Cell Locations

Adult stem cells are found in many locations throughout all of the human body.[11]

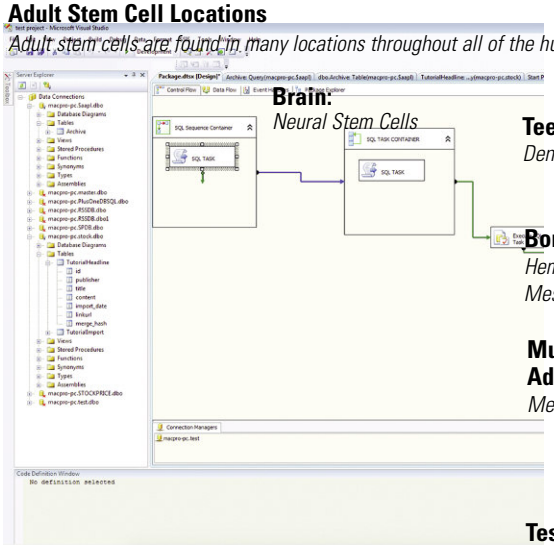


Figure 2

Blastocyst

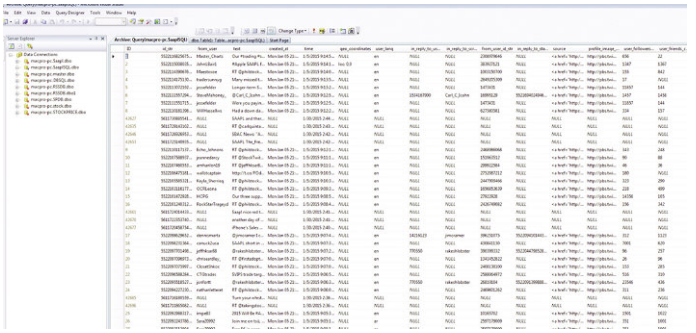
The circular clump of cells is a blastocyst and the rectangular shape is a needle head that is harvesting the embryonic stem cells from the blastocyst.[11]

A blastocyst is a group of cells that is one of the first stages of a mammalian embryo. The outer cells become the placenta and the inner cell mass becomes the embryo[6]. The placenta is an organ that connects a fetus to the wall of the uterus. It allows the fetus to obtain nutrition and dispel waste and gases through the mother's bloodstream. Embryonic stem cells are harvested from the inner cell mass of the blastocyst. The inner cell mass is illustrated in figure 3 below.

Figure 3

The Inner Cell Mass

The inner cell mass is a clump of cells that forms in the blastocyst. They are the inner most cells of the blastocyst. These cells will continue to differentiate to become a fetus[9].

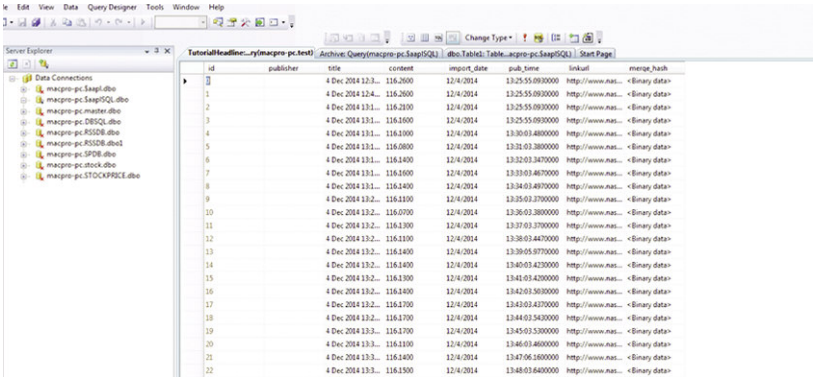


After the cells are harvested and stored in the lab they can be kept from differentiating. When the cells are kept separately they are unable to signal to each other and then will not be able to differentiate. But, if the cells do come together they form embryoid bodies, which are clumps of cells that can form when embryonic stem cells are kept in suspension[9]. When the embryoid bodies form they can start to spontaneously differentiate into any type of cell in the body[6]. This is shown in figure 4 below.

Figure 4

Differentiation

Embryonic stem cells from the inner cell mass can differentiate to become embryoid bodies. The embryoid bodies can form into any type of cell tissue[9].



Embryonic stem cells can be used to regenerate or repair diseased tissue and organs. Embryonic stem cells are totipotent or omnipotent meaning that one cell can differentiate into any type of cell. And the one cell can differentiate indefinitely producing many new cells[6]. There are limits on what can be done with these stem cells because of laws. Since some people believe that life begins before birth there are ethical issues of using embryonic stem cells because it is seen as destroying life[13]. However, there are many embryonic stem cells that are just thrown into the trash every day after abortions.

Perinatal Stem Cells

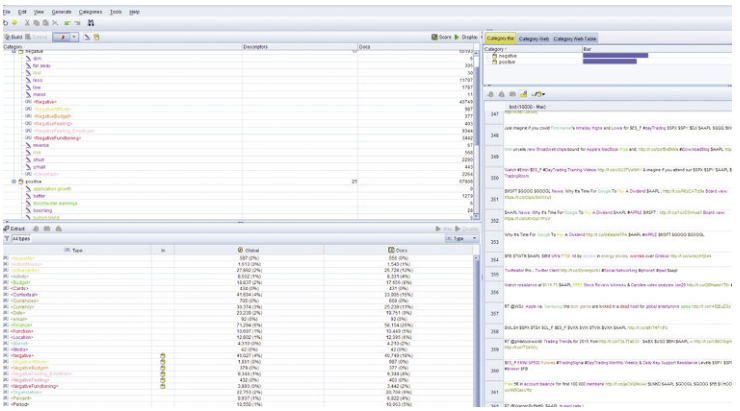
Perinatal stem cells are found in the amniotic fluid and umbilical cord blood. The amniotic fluid is what surrounds the fetus in the womb. It acts as a buffer to protect the developing fetus[10]. It contains electrolytes, aids for movement of the fetus, helps to develop the gastrointestinal tract, and promotes muscular and skeletal development.

Perinatal stem cells have the ability to change into any type of specialized cell[5]. The laws concerning the use of perinatal stem cells are less controversial[13]. However there are still

Figure 5

Umbilical Cord Stem Cells

There are two types of stem cells within the umbilical cord. Within the umbilical cord tissue there are mesenchymal stem cells and within the cord blood there are hematopoitic stem cells[1].



issues with using the cells because these stem cells can still be used for cloning. These cells can be collected during birth. Cord blood can be collected from the umbilical cord after it is cut.

There are two types of umbilical stem cells, cord tissue and cord blood. Cord tissue comes from the umbilical cord. And cord blood is the blood in the umbilical cord[1].

The stem cells in cord blood are collected after the umbilical cord is cut from a baby. These cells can be stored at a blood bank[5] so they can be used later by the baby, siblings of the baby and anyone that is a match.

Cerebral Palsy

Cerebral palsy is a disorder that is caused by damage in the developing brain. Brain damage occurs in one or multiple areas of the brain that are used for motor abilities[3,7].

The four main causes of brain damage for people diagnosed with cerebral palsy are periventricular leukomalacia (PVL), hypoxic-ischemic encephalopathy (HIE), intraventricular hemorrhage (IVH), and cerebral dysgenesis. PVL is damage to the white matter of the brain, HIE is lack of oxygen to the brain, IVH is a brain hemorrhage, and cerebral digenesis is a brain malformation and abnormal brain development[3,8].

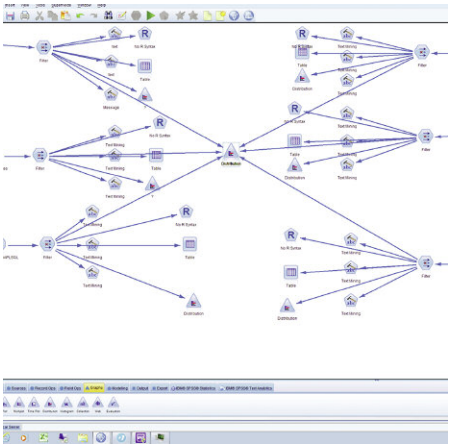


Figure 6

Cerebral Palsy

Cerebral palsy is caused by brain damage in many parts of the brain[2].

Case Study

Patient A

Patient A is a 13-year-old who has been diagnosed with cerebral palsy and a seizure disorder. Patient A suffered from hypoxic-ischemic encephalopathy during labor and had a loss of oxygen for about an hour. There were no complications during pregnancy and at 40 weeks gestation Patient A was born weighing 8.15 lbs. During labor, Patient A got caught in the birthing canal where Patient A suffered from a lack of oxygen for about an hour. After birth, Patient A was put on Phenobarbital and Topamax for Patient A's seizure disorder. But, both medications were discontinued. Patient A was put on Trileptal and is still on that medication currently for seizures. Patient A also began receiving physical therapy, occupational therapy, and speech therapy to improve physical and speech abilities.

As part of an elective experimental study on March 17, 2009 Patient A received an auto-reinfusion of Patient A's cord blood stem cells (hematopoitic cells) at the Pediatric Blood and Marrow Transplant Program at Duke Medical Center. Patient A received 5.89X10e8 TNC containing 2.42X10e7 cells/kg and a CD34+ count of 2X10e4 cells/kg.

Before the stem cell auto-reinfusion Patient A had achieved head balance and control by 3-4 months, sat independently at one year, began to pull to a stand at 8-9 months, and began walking independently for very short distances at 4.5 years old. Patient A also had very limited speech ability and was only able to communicate through about 5 distinct words and sounds. But, after the stem cell auto-reinfusion Patient A can walk long distances independently, walk up to 3 miles using a reverse walker, ride an adaptive bike for about 3.5 miles, and can communicate using 10 signs that are either made-up or from American Sign Language and 10-20 distinct words.

Currently Patient A is taking Trileptal and is on a ketogenic diet, which is a high protein and low carb diet in order to minimize seizures, and has continued to receive physical, speech, and occupational therapy. In addition to all of these treatments, Patient A is receiving hypobaric oxygen therapy. In hypobaric oxygen therapy Patient A is put in an oxygen tank.

Hypobaric oxygen therapy works by providing the patient with higher levels of oxygen by increasing the oxygen count in an oxygen chamber. High levels of oxygen help to stimulate the brain to improve overall brain function. It is important for Patient A to have hypobaric oxygen therapy because of the stem cell treatment. Like all cells, stem cells need the right environment to grow and differentiate. In order for there to be the right environment stem cells need oxygen.

Patient B

By comparison, Patient B is a 13-year-old diagnosed with cerebral palsy. Like Patient A, Patient B's cerebral palsy was also due to hypoxic-ischemic encephalopathy during labor. Patient B used to have a seizure disorder but no longer has seizures. Patient B cannot move independently and can only move around using a wheelchair and needs assistance for moving out of the chair. Patient B also has limited speech ability and suffers from bad muscle control. Patient B is currently taking medication for muscle control, and receives speech, physical, and hypobaric oxygen therapy. Patient B did not receive any stem cell treatment.

In conclusion, stem cells do affect the development and advancement of children with cerebral palsy. After Patient A's stem cell treatment Patient A has made marked improvement following Patient A's stem cell reinfusion. And Patient A is still continuing to make improvements today. But, Patient B did not make that much improvement except for getting over Patient B's seizures. Patient A's major improvement is due to the stem cells because both Patient A and Patient B have received the same therapies at the same treatment centers and with the same therapists; but Patient B's therapy is not as affective as Patient A's therapy because of the lack of the stem cell reinfusion. For example both Patient A and Patient B receive hypobaric oxygen therapy but, Patient B's therapy isn't as affective as Patient A's therapy because when Patient A receives hypobaric oxygen therapy Patient A's brain is being oxygenated and it promotes growth and differentiation of the stem cells therefore making repairs to the brain. When Patient B has hypobaric oxygen therapy the brain is oxygenated but it is not as affective because no stem cells are making repairs to the brain.

Conclusion

Stem cells have the potential to treat many diseases. But, there are many limits to what research can be done with them. Fortunately, there are many stem cell studies still being done with people with cerebral palsy and stem cells today. Although the case study in this literature review showed that cord blood stem cell reinfusions do improve the abilities of children with cerebral palsy there still needs to be more research done before it can be used regularly in hospitals as a non-experimental treatment. In most studies hematopoitic cells from the individuals are being used for research because it is more ethical and not as controversial[13]. These studies consist of patients that are different ages, patients that have treatment closer or further away from diagnosis, and patients with different causes of cerebral palsy.

In addition to cerebral palsy there is also research that is being done on how stem cells might be able to treat spinal cord injury, lung injury, stroke, Parkinson's disease, Alzheimer's disease, peripheral artery occlusive disease, liver disease, heart repair, bone repair, wound healing, and rheumatoid arthritis[12].

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A Comparison of the Antimicrobial Activity of Silver Nano-particles Against Antibiotics to Counter Antibiotic Resistance

by Sunny Birdi

Abstract

The efficacy of using silver nanoparticles as an alternative to antibiotics was investigated in order to limit the increase of antibiotic exposure, thus addressing the issue of antibiotic resistance in today's society. Lawns of *Escherichia coli, Staphylococcus epidermidis,* and *Bacillus cereus* were grown on soy agar plates in lysogeny broth. Filters containing different concentrations of silver nanoparticles and different types of silver crystals were placed on the agar plates. Similarly, antibiotic filters were placed on separate agar plates with the same species of bacteria, which served as a method of comparison for the effectiveness of silver as an antimicrobial agent. After a 24 hour period, "halos", which are zones of inhibition and evidence that bacterial growth was prevented, emerged around both filters containing silver nanoparticles and antibiotics. The diameters of these halos were measured as a way to compare the effectiveness of silver nanoparticles against antibiotics, and to consider the possibility of silver nanoparticles replacing antibiotics. Silver nanoparticles proved to be effective compared to certain antibiotics, yet most antibiotics prevented greater bacterial growth. However, silver nanoparticles definitely have the ability to fight off bacterial/microbial infection, and future research needs to be conducted to evaluate at what amounts of silver nanoparticles does silver function with equal effectiveness as antibiotics.

Introduction

Humans have been evolving on this Earth over millions of years. We are the prime example of evolution as we evolved from primates to full-fledged *homo sapiens.* However, now as we continue to evolve in subtle ways and our evolutionary pace has slowed down, humans are a major factor in the rapid growth and evolution of bacteria. According to a study conducted by The Centers for Disease Control and Prevention, 4 out of 5 Americans have been prescribed antibiotics[1]. The common belief in society is that by using antibiotics, harmful bacteria are eradicated from our systems and their growth is controlled. In reality, because of the great dependency we have on antibiotics, many strains of bacteria have developed adaptations against the antibiotics, which is known as antibiotic resistance.

This resistance in bacteria develops because the efficacy of antibiotics isn't always 100%, meaning it won't always kill of all of the bacteria in our bodies. In fact, a small number of bacteria survive the effects of the drug. These bacteria simply adapt antibiotic resistance

because of repeated exposure to antibiotics. Once this small sample of bacteria survives, it begins to reproduce and it spreads through the body again. This process is known as natural selection. Natural selection is the idea that organisms will continue to pass down or inherit traits that allow for their greatest chance of survival and reproduction. To reiterate, antibiotics can only kill about 98% of the harmful bacteria in our body, which means there's a small amount of bacteria left over that have adapted traits to prevent the antibiotic from killing them. Thus, by using antibiotics we constantly create bacteria that survive the treatment, adapt, and reproduce. This means that same antibiotic you used before may not be of any help in the future, because this new strain of bacteria has evolved and developed adaptations to how the drug damages those bacteria. Many antibiotic drugs are enzyme inhibitors, meaning they target or inhibit the activity of an enzyme which a pathogen relies on to run a reaction. Some of those adaptations are changes in its membrane composition so the drug cannot bind to it or changing the target-enzyme binding site, which an antibiotic would use to detect the bacteria. Thus with a modified target-enzyme, bacteria can easily conquer the antibiotic.

Today, researchers and doctors face an immense challenge as they have to modify their antibiotics against bacteria that are one step ahead of them, ready to evolve and adapting at a much faster rate than new antibiotics can be produced. A *New England Medicine Report Editorial* stated that we haven't needed this heightened development of antibiotics, since the 1930s[2]. We as humans are in a long-winded race against evolving bacteria, while bacteria continue to lap us.

Antibiotics have contributed a major source of treatment in society; however with the overuse of antibiotics it is imperative that society weighs the positives and negatives of our use of antibiotics. Antibiotics fight diseases, such as *pneumonia,* a bacterial infection in lungs, which were once life-threatening. Essentially, antibiotics help us recover from various bacterial infections and are easy to administer, either orally or by vaccine. There are also probiotics, which are live bacteria that benefit the human body, such as bacteria along our intestinal tract that assist in breaking down food and digestion. Humans have a special relationship with these bacteria; in fact humans have 10 times more bacterial cells on their bodies than human cells and 100 times more bacterial genes than human genes. Nonetheless, antibiotics specifically target the harmful diseases inducing bacteria in our body, and create resistant bacteria through natural selection. Overall, Valerie Reyna, a phycologist working at Cornell University, has pointed out many patients opt for antibiotics out of belief that they will feel better. She emphasizes this psychological notion that antibiotics will make one recover rapidly is embedded in society, supported by the fact that so many patients request prescriptions for antibiotics[9]. Consequently, people are increasing their dependency on antibiotics, and these patients reflect the larger society as well.

Although antibiotics do have positive effects on our health, an overuse of these drugs is one reason why we should cut back on taking them. In fact, if we continue to use antibiotics a greater variety of drug-resistant bacteria will emerge. *Methicillin-resistant Staphylococcus aureus* (MRSA) is a drug-resistant bacterium, which is related to a very common family of bacteria, Staph. This bacterium can cause severe infections on the skin, the urinary-tract, or the bloodstream, and is now resistant to over six commonly used antibiotics. Researchers note that even as new antibiotics are being developed to treat this bacterium, it continues to adapt and become resistant to treatment. Richard Lenski, a Michigan University professor, has carried out his own experiment using *E.Coli* bacteria. He grows species of *E.Coli*, and observes how they

utilize both glucose and citric acid for cell work and energy. After he observes the colony of bacteria, he freezes the colony and continues growing *E.Coli* on the agar plates, and he has been conducting this experiment since 1988. Over the course of 26 years, Lenski has observed that after 20,000 generations of the bacteria, 45 mutations had taken place on the original 4,000 ancestral genes[11]. The bacteria had begun to use glucose differently, and started depending on citric acid for energy instead. Lenski has grown over 59,000 generations of 12 different strains of *E.Coli* and he can attest to the fact that bacteria evolve at a scary and rapid pace. Again, because so many Americans and people around the world are prescribed antibiotics, as we continue to use antibiotics this evolution of bacteria will continue.

If strains of bacteria continue to become drug-resistant, one option can be producing stronger antibiotics, yet those could also carry strong side-effects, including severe headaches or nausea. This has led to critical research being conducted on how to prevent bacteria from becoming drug-resistant and a search for an alternative to traditional antibiotics. Researchers are now investigating the different layers behind antibiotic resistance. Professor Vincent A. Fischetti, head of the Laboratory of Bacterial Pathogenesis and Immunology, is observing the bacterial armor of drug resistant *Staphylococcal* strains. Specifically, he is looking at the bacterial structure of *Bacillus anthracis,* which is a bacteria known to cause anthrax—a lethal disease that targets the digestive tract and central nervous system. Professor Fischetti is using bacteriophages. Bacteriophages are viruses, foreign DNA, enclosed in a coat of protein. These bacteriophages have helped researchers view the weak spots in the bacterial armor, by observing at which sites bacteriophages could invade the membrane of the bacteria. This has allowed Professor Fischetti to come up with a new type of antibiotic that targets these same pathways, but also he has gained insight on how to limit enzyme-inhibiting drugs from promoting antibiotic resistance. Through his research, it became clear that *Bacillus anthracis* was relying on a bacterial-enzyme called 2-Epimerase to create a protective cell wall around its cell membrane. This cell wall prevents antibiotics from directly binding to the bacterial membrane of the bacteria and renders it ineffective. Along with his colleagues, Professor Fischetti studies the binding site of this 2-epimerase enzyme and its crystalline structure, researching where this enzyme would bind to on the bacteria. He noted that this enzyme was activated by a substrate binding to an allosteric site on the bacteria. The allosteric site is another binding site on the enzyme that can regulate the rate of certain reactions, in this case the formation of a cell wall. Thus, he developed inhibiting compounds which would target the allosteric site of the enzyme, preventing its activation and the formation of a bacterial cell wall. This antibiotic is now known as Epimerox, and Professor Fischetti has conducted several studies to show that bacteria are not developing resistance to this drug at all[5].

Epimerox doesn't necessary kill the bacteria entirely, it inhibits the use of the 2-epimerase target enzyme by *Bacillus anthracis* and since it can't create a protective cell wall, the bacteria are very susceptible to other factors. Resistance doesn't develop because this antibiotic disrupts the mechanism used for resistance, whereas previously antibiotics would be ineffective against bacteria that could form a cell wall. Many researchers are following Professor Fischetti's research and exploring the different mechanisms bacteria use to develop this resistance, yet bacteria are evolving much faster than these new antibiotics.

However, Professor Fischetti's research still focuses on creating antibiotics to counter the rising species of bacteria, which could create simply another dependency on novel antibiotics. Furthermore, there is also research being done that strays away from the traditional use

of antibiotics, and focusing on other antimicrobial factors that we can possibly use to substitute antibiotics. This leads me to the research I'm currently conducting using colloidal silver (silver nanoparticles diluted in distilled water) and its antimicrobial effect on the bacteria strain *Staphylococcus Epidermidis, Escherichia Coli,* and *Bacillus Cereus*.

Silver has long been known to have antimicrobial properties against microorganisms. In history, previous civilizations have used steel materials to store food or water. 3,000 years ago, Phoenicians used bowls made of silver, while Romans later used silver to make cups. Ancient civilizations relied on silver to prevent the growth of any fungi or mold on their food, thus keeping their food or water supply fresh and bacteria-free[3]. Even today, we use silver-made eating utensils such as silver knives or forks. This antibacterial property of silver is also known as the oligodynamic effect. The oligodynamic effect is a general term used to describe how and why silver has antimicrobial properties. Most research evidence agrees that the reason silver is known to have an antibacterial effect is due to its high toxicity towards microorganisms. Silver can exist in ionic form. Its ions can denature enzymes of the target cell, and these ions can possibly react with other functional groups of the bacteria. These different factors disturb bacteria, leading to bacteria dying or at least inhibiting growth.

One way scientists have measured the antimicrobial effect of different antibiotics is through the use of the Kirby-Bauer assay. The Kirby-Bauer assay is a test that uses small filters that contain antibiotics, and these filters are placed on a lawn of bacteria grown on agar plates. Agar plates are petri dishes containing nutrient media that support the growth of bacterial culture, composed of a layer of polysaccharide agarose gel. After the filters are placed on this lawn of bacteria grown in agar plates, the plates are placed for incubation overnight. After the incubation period, one can observe the agar plates for zones of inhibitions around the filters. Zones of inhibition are regions of clear "halo"-like space which forms around the filter. These blank spaces among the lawn of bacteria indicate that bacteria were not able to grow around those filters. Furthermore, one can measure the diameter of each "halo" around each filter, and compare their measurements to a chart with standard values and verify whether their antibiotic filter was truly effective. Although the Kirby-Bauer Assay is commonly used in several studies to test the strength of antibiotics, I will be using this assay to observe how well silver can be used to prevent bacterial growth in comparison to antibiotics.

Few articles have been published that evaluate the effect of silver nanoparticles as an antimicrobial agent[6,8,10], however most articles support the use of silver as a bactericide. All of these researchers tested the antibacterial properties of silver by placing a measured amount of silver nanoparticles directly onto the bacteria, and then observed for growth of bacteria. In this study, I placed filters containing varying amounts and types of silver compounds onto the bacteria, and observed for inhibition of growth by the bacteria. Thus, I emulated the Kirby-Bauer assay and placed filters of different concentrations of silver on the different strains of bacteria.

The three bacteria grown in this experiment were *Escherichia coli, Staphylococcus epidermidis,* and *Bacillus cereus.* All bacteria are broken down into categories of gram-negative bacteria or gram-positive bacteria. The difference between the two types is that gram-negative bacteria have a thicker peptidoglycan membrane or cell wall, so because of their impenetrable membrane it has greater antibiotic resistance. *E.Coli* is a gram-negative type bacteria, while *Staphylococcus epidermidis* and *Bacillus cereus* are gram-positive type bacteria. By conducting a Kirby-Beaur Assay using silver compounds, I was able to measure the effect of silver on a

wider variety of bacteria and compare the effects of silver on the growth of both gram-positive and gram-negative bacteria.

Materials and Methods

As stated earlier, the purpose of my experiment/method was to compare the effect of silver to antibiotics as antimicrobial agents on the different species of bacteria. My experimental group would be the Kirby-Bauer assay of filters containing different forms of silver, placed on the three different species of gram-positive and negative bacteria. The experiment entailed the preparation of bacteria and growing bacteria on nutrient agar plates, as well as the preparation of silver compounds and filters containing silver. The antibiotic filters: *Ampicillin (10 ug), chloramphenicol (30 ug), gentamycin (10 ug), penicillin (10 ug), streptomycin (10 ug),* and *tetracycline (30 ug)* were purchased and already manufactured.

Starter bacteria cultures samples containing *E.coli, Staphylococcous epidermis,* and *Bacillus cereus* were rehydrated in Luria Bertani broth medium required for each bacteria, in a 1:10 dilution ratio. Six nutrient agar plates were also incubated for 24 hours at 37 degrees Celsius. Two agar plates were plated for each bacteria, thus creating two sets of plates with each species of bacteria. These two sets were used as the control group and experimental group: in the first set, three agar plates with each type of bacteria would have filters containing antibiotics, while the second set of three agar plates with bacteria would have filters containing silver or silver compounds.

Silver was prepared using a single replacement reaction with copper and silver nitrate. Pieces of copper wires were placed in tubes filled with .5 moles of silver nitrate, and silver formed on the strands of wires were collected. The reaction was allowed to continue until enough silver precipitate was collected. When the reaction was carried out using a greater volume of silver nitrate, more concentrated silver crystals were collected from the copper wires. Thus, two different types of silver were used in this experiment—dark precipitate silver and purified crystalline silver—and both types were placed in separate Eppendorf tubes. Three blank filters were placed in these Eppendorf tubes so they could absorb the concentrations of silver from each tube. They were left in the Eppendorf tubes for 24 hours.

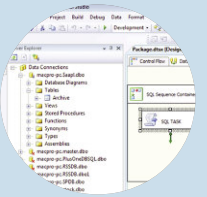
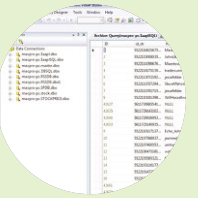
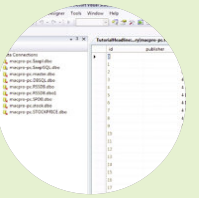
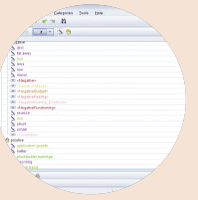
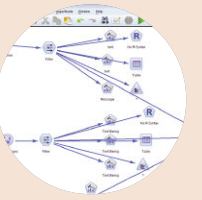
Another set of three filters were prepared containing 40 micrograms of silver nitrate. Silver nitrate was used, so we could observe for an antimicrobial effect of another silver compound. A fourth set of three filters containing 40 micrograms of colloidal silver (500 ppm) were also prepared. In total, these four sets of filters would be placed on three agar plates containing the three species of diluted bacteria *(Escherichia coli, Staphylococcus epidermidis,* and *Bacillus cereus),* serving as the experimental group. Each of these three petri-dishes was divided into five equal sections like a pie-graph. After the lawn of bacteria on the agar plates had become a solid gel, five filters, containing 40 ug colloidal silver (500ppm), 40 ug silver nitrate, silver precipitate, purified silver participate, and one blank filter were placed on each of the three plates. Each filter was firmly pressed onto the solid gel of the three separate plates containing *Escherichia coli, Staphylococcus epidermidis,* and *Bacillus cereus* species of bacteria.

After the experimental group was completed, the control group was set up. The remaining three agar plates were divided into seven equal sections, each section served as the space for one antibiotic. One filter of each antibiotic *(ampicillin, chloramphenicol, gentamycin, penicillin, streptomycin,* and *tetracycline)* was placed on the second set of agar plates, and firmly

pressed down on the solidified gel. Once both the experimental group and control group were prepared, the six agar plates were placed in an incubator for 24 hours. After this incubation period, all of the plates were removed and observed for clear zones of inhibitions. Once the zones of inhibitions were observed, the diameter of each of these zones in the section were marked and measured.

Data

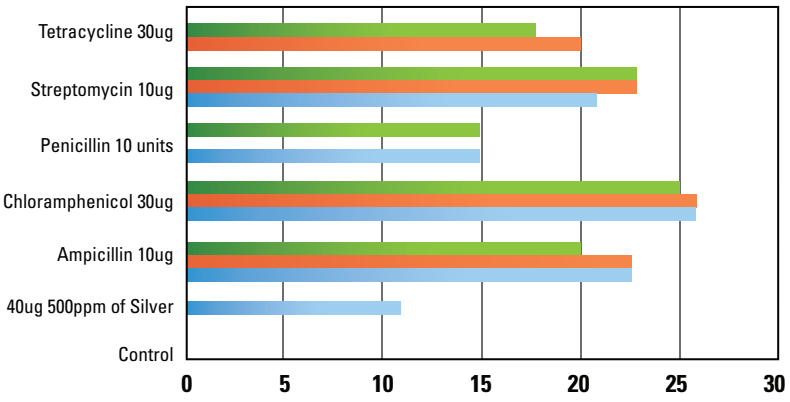
Figure 1a-f

Strain of Bacteria	Treatment with Anti-Biotic Filters	Treatment with Silver Filters
E.Coli	 A	 D
Bacillus Cereus	 B	 E
Staphylococcus Epidermis	 C	 F

All photographs show filters placed on agar plates with bacterium. Figures 1a-c show the zones of inhibitions for the six antibiotic filters following the first two control filters: Ampicillin (10 ug), chloramphenicol (30 ug), gentamycin (10 ug), penicillin (10 ug), streptomycin (10 ug), and tetracycline (30 ug) in the clockwise direction. Figures 1d-f show zones of inhibitions for silver filters: control filter, 40 µL of 500 ppm colloidal silver, 40 µL of silver nitrate, disk containing dark precipitate silver, and crystalline silver on each strain of bacteria in the clockwise direction.

Figure 2

Inhibition of Bacterial Growth by Antibiotics

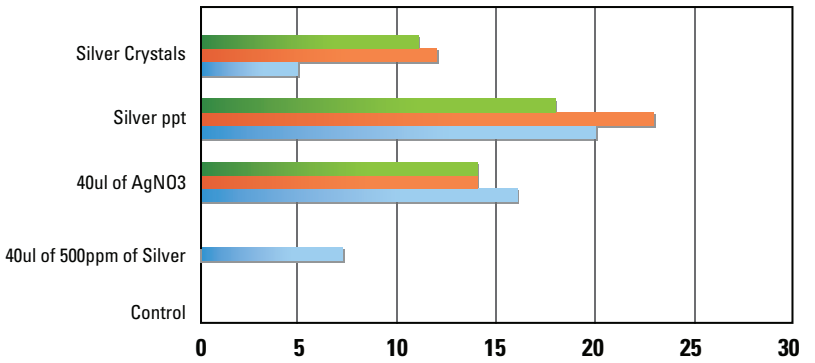


	Control	40ul 500ppm of Silver	Ampicillin 10ug	Chlorampenicol 30ug	Penicillin 10 units	Streptomycin 10ug	Tetracycline 30ug
B.Cereus	0	0	20	25	15	23	18
E.Coli	0	0	23	26	0	23	20
S.Epi	0	11	23	26	15	21	0

Left is a horizontal bar-graph, for which the Y-axis (independent variable) is the type of antibiotic filter placed on one of the petri-dishes, while the X-axis (dependent variable) is the length of diameters for zones of inhibition in millimeters. Below the graph is a table with the lengths of zones of inhibitions (mm) of each antibiotic filter placed on the three species of bacteria: Escherichia coli, Staphylococcus epidermidis, and Bacillus cereus.

Figure 3

Inhibition of Bacterial Growth by Silver Compounds



	Control	40ul 500ppm of Silver	40ul of AgNO3	Silver ppt	Silver Crystals
B.Cereus	0	0	14	18	11
E.Coli	0	0	14	23	12
S.Epi	0	7	16	20	5

Left is a horizontal bar-graph, for which the Y-axis (independent variable) are the silver compounds placed on the three types of bacteria, while the X -axis (dependent variable) is the length of diameters for zones of inhibition in millimeters which resulted on the bacteria. Below the graph is a table with the lengths of zones of inhibitions (mm) of each silver compound placed on the three species of bacteria along with a blank disk for control.

Results

Several zones of inhibitions were visible on the petri dishes as shown in Figure 1a-1f. As shown in Figure 1, clear zones of inhibitions had formed around the agar plates containing antibiotics and plates containing silver compounds. A lawn of *E.Coli* was grown on both of these plates. The *E.Coli* treated with silver compounds had zones of inhibitions around the filter containing 40 μL of silver nitrate, dark precipitate silver, and crystalline silver. The lengths of the diameter for the zones of inhibition were 14 mm for the silver nitrate, 23 mm for the dark precipitate silver, and 12 mm for the crystalline silver filter. Silver compounds also induced zones of inhibitions in the *Staphylococcus epidermis* bacteria, and clear zones of inhibitions were present. Similarly to *E.Coli*, zones of inhibitions in the *Staphylococcus epidermis* bacteria formed around filters containing 40 μL of 500-ppm colloidal silver, 40 μL of silver nitrate, dark precipitate silver, and crystalline silver. The diameters of the zones of inhibition on the *Staphylococcus epidermis* bacteria strain measured 7 mm for the 500-ppm of colloidal silver filter, 16 mm for the filter containing 40 μL of silver nitrate, 20 mm for the filter containing precipitate silver, and 5 mm around the crystalline silver placed on the agar. Zones of inhibitions were also present in the third bacteria strain *bacillus cereus*. The diameters of the silver measured accordingly: 14 mm in length for the filter containing 40 μL of silver nitrate, 18 mm in length for the filter containing 40 μL of dark precipitate silver, and 11 mm around the crystalline silver.

In the control group, Figures 1a-c, zones of inhibitions also formed around certain antibiotics across the three strains of bacteria. The lengths of diameters are all recorded in the chart under Figure 2b. Every antibiotic created a zone of inhibition-measuring at least 15 mm in length on all three strains of bacteria, except the filter containing *penicillin* did not induce a zone of inhibition on the *E.Coli* bacteria and *tetracycline* did not create a zone of inhibition on the *Staphylococcus epidermidis* bacteria.

Discussion

The data and results provide evidence for the efficacy of silver as an antimicrobial agent. In addition, I was able to test the strength of silver as an antimicrobial agent by comparing the diameters of the zones of inhibitions formed by both of the filters containing silver compound and antibiotic filters.

Filters containing 40 μL of silver nitrate and dark precipitate silver, collected from the single replacement copper reaction, induced zones of inhibitions with the greatest diameter. The zones of inhibitions formed by silver nitrate and the dark precipitate filter can be observed in Figure 1d-f. By comparing the photographs from each row in Figure 1a-f, it can easily be observed that the zones of inhibitions that surround the filter containing silver nitrate or silver precipitate match the size in diameter of certain antibiotics placed on the same bacteria. Filters containing silver nitrate successfully prevented bacteria growth in all three types of bacteria, evident by the clear zones of inhibition. Looking at the table in Figure 2b, the length of inhibition zones for silver nitrate varied from 14 to 16 mm for all three species bacteria. This is comparable to the length of inhibition zones for antibiotic filters, specifically the efficacy of penicillin on both *Staphylococcus epidermidis* and *Bacillus cereus*, which also had inhibition zones of 15 mm. It's evident from the results that silver nitrate was able to prevent growth on all three species of bacteria despite being gram-negative or gram-positive. This demonstrates that silver nitrate

is an effective antimicrobial agent, yet more research needs to be conducted in order to clarify which element in the compound causes this antimicrobial effect. Nitrate alone is not commonly known to prevent bacterial growth, but—as shown by the fourth filter containing dark silver precipitate—silver alone does inhibit bacterial growth.

The filter containing silver precipitate from the single replacement reaction between copper and silver-nitrate created the largest zones of inhibitions (in diameter) compared to the other filters in the control group and even some antibiotic filters. The lengths of the inhibition zones for the filters containing silver were equal to or greater than the zones of inhibitions created by *ampicillin, penicillin, streptomycin,* and *tetracycline.* This is significant because certain antibiotics such as *penicillin* were not able to prevent any growth of the bacteria. This is plausible in the case of penicillin because it was unable to prevent growth of *E.Coli*, which is a gram-negative bacteria and is less receptive to antibiotics, due to the outer membrane. *Penicillin* also had the smallest zones of inhibitions out of all of the antibiotics used in the control group, which is credible because it is one antibiotic that most MRSA bacteria have developed resistance against. *Tetracycline* was another drug in the experimental group which caused no zones of inhibitions for a type of bacteria, specifically, *staphylococcus epidermis. Tetracycline* has been in use since the 1940s and perhaps its inability to prevent bacterial growth suggests that strains of *staph* bacteria are no longer affected by it.

The type of bacteria that the filters were placed on did affect the antimicrobial effect of many antibiotics. The data shows that antibiotics were able to prevent greater amount of growth in the *Staphylococcus epidermidis* and *Bacillus cereus* bacteria, since both recorded longer inhibition zones. This data makes sense because *Staphylococcus epidermidis* and *Bacillus cereus* are both gram-positive type bacteria, thus have a thinner outer- membrane in comparison to the *E.Coli*. The thinner membrane of these gram-positive type bacteria makes it susceptible to antibiotics, resulting in greater inhibition of growth.

Thus, it is critical and significant that silver compounds, whether it be silver nitrate or precipitate silver, were able to inhibit bacterial growth, despite being placed on agar plates with gram-negative or gram-positive bacteria. MRSA bacteria have become resistant to the effects of penicillin, so if silver were able to be utilized in an efficient manner, it could replicate the efficacy of penicillin, and perhaps even prevent greater bacterial growth, shown by the data. The filters containing silver nitrate and precipitated silver were best able to emulate the effect an antibiotic would have on the these three species of bacteria. I believe future research needs to be conducted to determine if, perhaps, soaking the antibiotic filters in silver precipitate would result in greater zones of inhibitions while also causing less development of drug-resistance bacteria. Through this study it has become evident that silver does have an antibacterial property, thus it would be interesting to investigate whether that property could enhance the effect of antibiotics. Another solution to antibiotic resistance could be further investigating silver as an antimicrobial agent in order to replace the usage of antibiotics, since it is evident silver can prevent a similar amount of growth compared to antibiotics. More research also needs to done to determine how to quantify the concentration of silver precipitate and measure at what concentrations the silver nitrate or precipitate would be most effective.

Other filters tested in the experimental group yielded some type of antimicrobial effect. The filters containing 500 ppm colloidal silver resulted in very small zones of inhibitions, and were unable to prevent bacterial growth. The control filter resulted in no prevention of bacterial

growth and successfully functioned as a negative control. The filters containing 40 μL of colloidal silver only prevented minimal growth in *Staphylococcus epidermidis* colony, and had an inhibition zone of 7 mm. Further research needs to be conducted to determine at what amount colloidal silver can be more effective and prevent more bacterial growth. Colloidal silver is a much smaller molar form of silver and was not as effective on the bacteria, whereas silver precipitate was able to prevent a much greater amount of growth at a larger quantity. On the other hand, the fifth filter containing the purified crystal silver precipitate prevented a small amount of bacterial growth, but not as significant as the filter containing less purified silver precipitate. Since the crystalline silver formed from the single replacement reaction was directly applied onto the agar, this could mean that if silver is too large it could also be ineffective. Moreover, a balance needs to be reached to decide at what molecular size and concentration silver or possibly silver nitrate can inhibit a greater amount of bacterial growth.

I believe more research also needs to be conducted on how exactly the silver reacts with the bacteria to prevent the bacteria from growing. Previous literature suggests that the toxicity of silver disrupts the activity of the enzymes along the membrane of bacteria[8,10]. Other literatures also suggest the free radical ions of the silver react with the charges with in the bacteria to have a similar effect that destroys the cell membrane of the bacteria. Although it might be difficult, it would be helpful to observe how the silver interacts with the bacteria at a microscopic level to gain a better sense of how the silver particles react with the bacteria.

Scientists are still discovering novel methods to fight against MRSA and drug-resistant bacteria and recently published studies have discovered a new toxin named, teixobactin, which is an organic antibiotic found in soil[7]. Teixobactin works by targeting parts of the bacterial cell wall that cannot mutate, thus even if bacteria mutate they will not be able to prevent the binding of this antibiotic to its membrane. The door is still open for further discoveries. Quorum sensing is another reasonable solution to antibiotic resistance, and it specifically inhibits bacteria from sending chemical signals known as auto inducers to one another. Quorum sensing targets the inhibition of bacterial communication, and perhaps intercepting how bacteria communicate with one another can prevent the formations of new mutations or possibly growth[4]. I believe with research being conducted on nanotechnology, medicine is also adjusting to this trend and molecular compounds such as silver can be implemented as nano-medicine.

In conclusion, through the several trials conducted in this experiment, different quantities and forms of silver were able to prevent bacterial growth. Filters containing silver precipitate were even able to inhibit as much growth as some of the commonly used antibiotics tested in the control group. I believe to some degree the possibility of silver as an alternative to antibiotics is likely in the future once we gain a better understanding of how exactly the silver reacts with bacteria and at what quantities is it most effective. The bacterium in this experiment were also diluted, thus one also needs to consider if silver would be yield proportional zones of inhibitions in a less diluted sample of bacteria. A solution to antibiotic resistance is an important concern in today's world of medicine, health, and society.

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Zebrafish as a Reliable Model for Alzheimer's Disease

by Alexandra Pachter

Abstract

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative diseases today and affects about 5.1 million Americans[1]. We know that the disease degenerates and kills brain cells that process, store, and retrieve information, but have yet to find the underlying cause of this destruction. One theory is the amyloid hypothesis, which states that accumulated microscopic amyloid plaques, which form from a build-up of the protein fragment called beta-amyloid, disrupt neurons by clogging the points of cell-to-cell connection and activate an inflammatory response that ultimately causes neuronal death[2].

Zebrafish have been adopted as a model for studying Alzheimer's disease[3]. In conjunction with other studies, I review the zebrafish model for its use as a physiologically relevant animal model that closely mirrors the human AD neuropathology. More specifically I compare proteins involved in the development of Alzheimer's such as APP (amyloid protein precursor), beta-amyloid toxicity, *PSen1,* and *PSen2,* in the human and zebrafish. I conclude that the zebrafish model is similar enough to the human model to accurately and efficiently be used in testing and predicting drug responses for treating the effects of Alzheimer's disease.

Introduction

In today's day and age, it seems as though all the world's mysteries are rapidly unraveling. With the help of innovative scientists and technology we've landed on a comet, mastered calculus, and have begun to unravel and better understand the brain system. Despite the deep indents cognitive and neurosciences have made in discovering the intricate makeup of the human brain and its functions, the cerebellum still remains one of the most inconclusive topics of our time. The complex system in our skull, filled with processes and synapses, is outstanding. Our brains make us consciously aware human beings who can rationalize, make decisions, and self-reflect. Due to this, we experience and react to our environment, forming and storing memories from our surroundings. However, science is still not able to pinpoint the specific mechanisms in the brain that clearly explain these experiences. The human brain still remains in many ways, a mystery. Synesthesia does not have any noticeable drawbacks. For most, it is seen as an extra sense. In some cases, it may even be an advantage. People with auditory-visual synesthesia have a much easier time remembering music notes because sounds elicit colors. For grapheme color synesthetes there is a similar advantage. Remembering the spelling of particular words

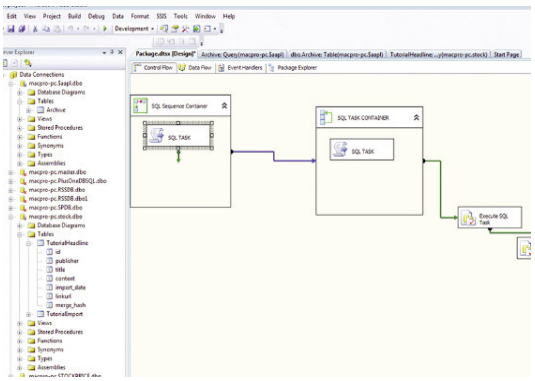
becomes especially easy if the synesthete can remember the progression of colors (Eagleman). A condition like synesthesia is very reminiscent of something much more common: optical illusions. Both induce a change in perception by manipulating the senses.

Fortunately, scientists are persistent in unraveling the brain's inner workings. Although studying the human *in-vivo* would obviously be the most direct path for understanding the brain, it is limited, as it brings up both ethical and technical problems involved in human manipulation4. Because of this limitation, animal models have been constructed to help explain brain function and its role in the human body[4]. Although their brains have similar basic structures and functions, the brains of animal stand-ins are not an exact biological replica of the complex human brain[4]. The innovative and interesting research lies in discovering the brain's functions. Non-animal methods such as computer modeling are somewhat effective, but do not allow for the same sort of breakthrough discoveries that *in-vivo* research might offer and they are not extensive enough to integrate all the potential variables that come along with studying the brain's functions *in-vivo*[4]. Because of these shortcomings, we turn to animal models to better grasp how the healthy human organism works[3]. Understanding a healthy organism is the key to understanding the body's mechanisms of disease.

Mice and rats are the two most commonly used vertebrates in research because they allow the scientist to easily manipulate and observe the genome[5]. The genes of rodents can be easily altered, which allows for scientists to knockout or knock-in and add whichever genes are in their specific focus. A knockout mouse or rat is an animal model. It has been genetically engineered so that a single gene is turned off or 'knocked-out'[5]. Deleting a gene blocks the function of a protein and tells us what is missing from the healthy model. From there, scientists can determine the particular gene's role in the model by observing the phenotypes of individuals lacking the gene[5]. Since mice and rats have the same number of genes as humans, and most of these genes in the genome are 85 percent similar to their related gene in the human genome, rodents are considered *complementary models*[4]. A complementary model must have the genetic makeup and expression similar enough to the first model. Mice and rats, as animal models, can work side-by-side with humans to enhance our understanding of human genes, proteins, and eventually, brain function.

Image 1

A laboratory mouse in which a gene affecting hair growth has been knocked out (left) is shown next to a normal lab mouse[24].



Seeing what happens in the knockout rodent model gives us an understanding of the protein's function in the human[4]. Genes in the DNA sequence are transcribed and expressed as proteins. Many genes specifically code for the proteins involved in aspects of neural cell development, such as the establishment of neural synaptic connections (cell-to-cell communication)[6]. Neurons are the brain's communication towers. They are a type of nerve cell specific to the brain and have two types: sensory and motor. Sensory neurons send signals to the brain and spinal cord, while motor neurons receive signals from the brain and spinal cord[6]. Like other nerve cells, they transmit information by electrochemical signaling. Neurons have signals

that later form memories and thoughts that move through individual nerve cells as a tiny electrical charge[6]. These nerve cells connect at synapses. When a charge reaches a synapse, it triggers a release of chemicals called neurotransmitters, which travel across the synapse and carry signals to other cells[7]. When I touch a hot stove, nerves in my fingers will shoot electric impulses along axons, and transmit the message of "hot" to the neurons in my brain, which will in

Image 2

Knockout Mice Model[25].

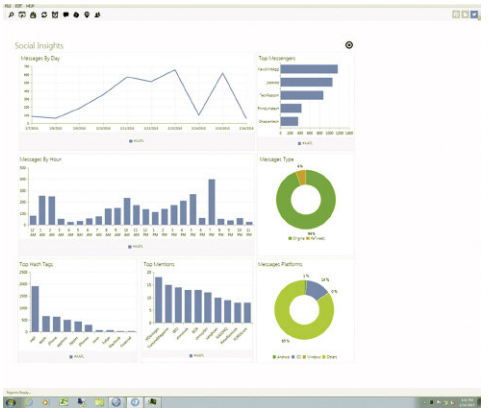
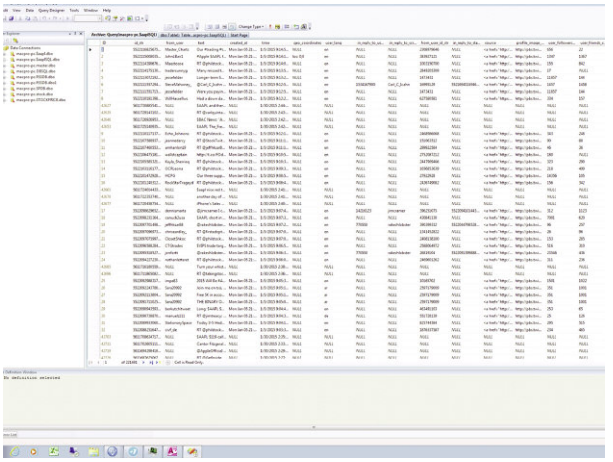


Image 3

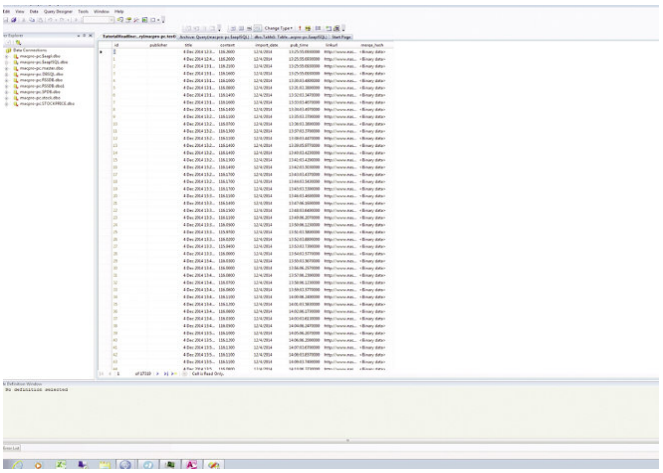
The procedure for making mixed-genotype blastocysts[6].



turn tell, through a synapse, the nerves in my body to react[8]. I will respond accordingly: feeling pain, quickly pulling my hand away, and maybe even screaming. Following the reaction, the neurons in my brain will continue to communicate and store the memory that hot objects are harmful. When there is something wrong with these processes, I could not react according to the "hot stove" due to blocked signals or death of neurons. Problems could arise from physical damage to my nervous system and my body. Your brain is the most powerful organ in your body and contains about 100 trillion of these neural synapse points[8]. Over the course of our lives, our experiences create patterns and strengthen our neural signals and connections. Each neuron in your brain is responsible for making your memories, skills, and essentially who you are[8]. When neuron cells die or lose connection from build-up of harmful proteins or old age, we can see damage to the brain and strange behavior[7].

Image 4

Cell signaling in a 'Neuron Forest'[7].



Alzheimer's Disease

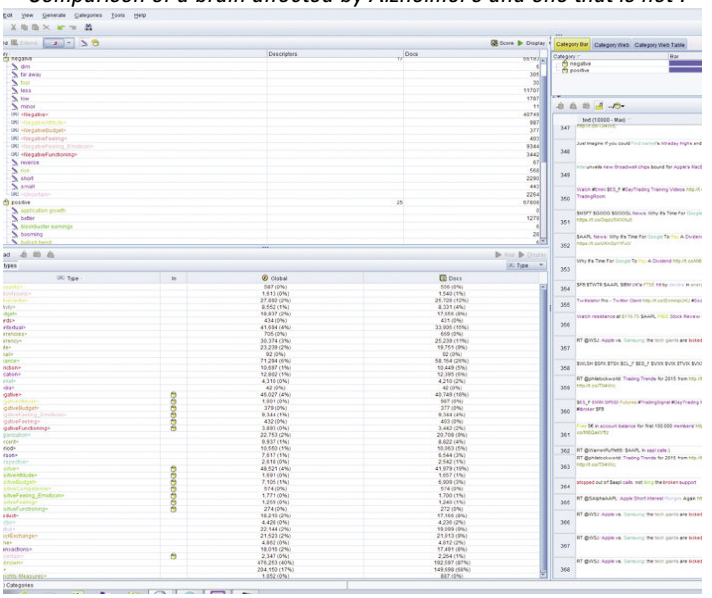
Alzheimer's is an irreversible and progressive brain disease where neurons and nerve cells are destroyed; the disease slowly damages memory and thinking skills, and eventually daily living becomes impossible[9]. Over time, toxic proteins clog the brain system, which leads to nerve cell death and tissue loss throughout the brain[7]. Because of lost matter, the brain shrinks dramatically and the disease affects nearly all of its functions[7] (See Image 5). Alzheimer's disease slowly progresses in five stages. The early, or preclinical stage begins long before any symptoms become noticeable and can sometimes last for decades. Although undetectable in behavior, image technology can identify beta-amyloid deposits[10]. The next stage is mild cognitive impairment, where the patient can see mild changes to their memory and thinking. Although relationships can still be functional, memory lapses can make conversations and appointments challenging[10]. People may forget easily memorable information such as recent events, have a harder time making coherent decisions, and misjudge the amount of time or steps needed to accomplish simple tasks. The three final stages are when the patient begins to

enter dementia[10]. Mild dementia is diagnosed when the patient has significantly clear trouble with memory and thinking. The person may experience misplacing belongings, getting lost in familiar places, difficulty problem solving (like balancing a checkbook), and changes in personality where they might become withdrawn or show uncharacteristic anger[10]. When the patient reaches moderate dementia, he or she may need help with daily activities and self-care. There is more poor judgment and confusion—losing track of the day of the week/season/month, inability to recognize their own belongings, and confusing strangers for family members. At this stage there is even greater memory loss, as toxicity and neuron death spreads to the hippocampus, the region of the brain that controls memories[9]. Patients may begin to have unfounded suspicions about friends and family—believing they are being stolen from or cheated on[10]. The final and most alarming stage of Alzheimer's disease is severe dementia. Mental function has significantly declined and physical capabilities are impaired. The patient may lose the ability to communicate coherently, require help with eating, dressing, and/or using the bathroom, and be unable to walk, sit, or hold up his/her head without help. Sometimes the patient may lose the ability to swallow and control bladder/bowel functions. At this stage, the patient experiences extreme memory loss and could exhibit fits of anger and confusion—bursts which are particularly alarming to the patient's loved ones[10].

Patients can live with Alzheimer's for as few as 3-4 years without knowing or 10 or more years, depending on the age in which the disease forms[10]. The impairment progression of complete loss of cognitive functioning, memory, and reasoning is directly linked to the neural degeneration that comes with age and toxic buildup in the body[7].

Image 5

Comparison of a brain affected by Alzheimer's and one that is not[7].

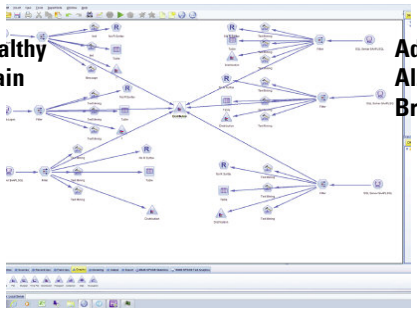


Complementary on the Genetic Level

The Human Genome Project, completed in April 2003 gave scientists the ability to read nature's blueprint for building a human being, or understand the codes on DNA strands[11]. Upon completion, the 26,000 or so genes that contribute to the human body were identified, but scientists would still need to pinpoint each gene's' function[11]. Complementary animal models are one of the ways in which scientists are continually discovering these gene's functions. About 70% of all human disease genes have functional homologs in zebrafish—as we can see in image 10[12]. And more importantly, of the genes causing disease in humans, 84% of them have zebrafish analogues, or similarities[12].

Image 6

The Alzheimer's brain shows a shriveled cortex (area of brain involved in thinking, planning, and remembering), shrinkage in the hippocampus (area of cortex important in forming new memories), and larger ventricles (fluid-filled spaces within the brain)[7].



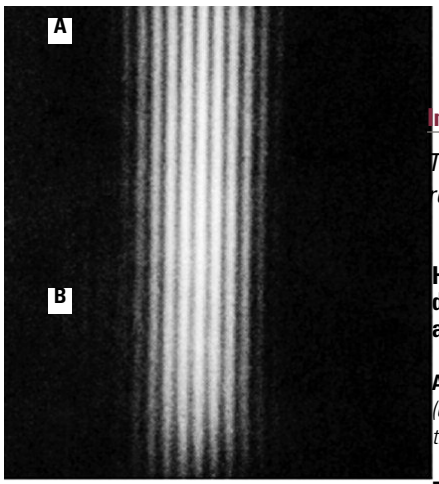


Image 7

The Zebrafish reference genome sequence and its relationship to the human genome.

How can we better assess the parallels and differences between Zebrafish (an NIH-approved model organism) and Humans?

A: *Overlap between species = number of orthologues (copies of gene, not genes themselves) at the time of their phylogenetic split.*

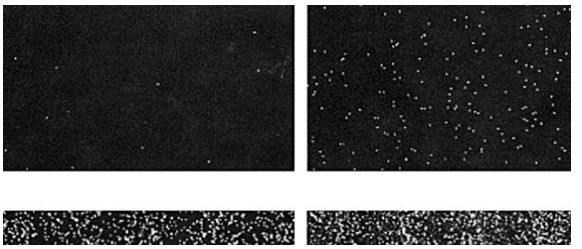
B: *Relationship among "ohnologues": TSD (teleost-specific genome duplication) - related genes.*

Zebrafish Research with Alzheimer's Disease

Zebrafish have become more popular as complementary animal models because they are cheaper to maintain, reproduce much faster than rodents, and are transparent. Scientists have been opting to study zebrafish, instead of other animal models, for their function in explaining neurodegenerative diseases on the molecular and behavioral level[11].

Image 8

A zebrafish[27]



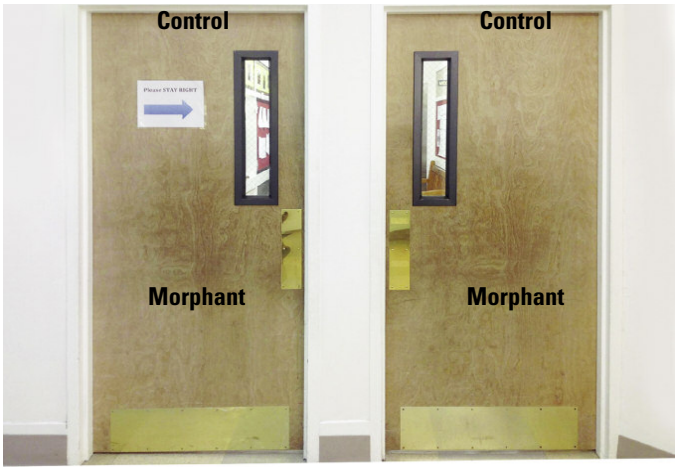
Zebrafish, or *Danio Rerio,* have become one of the preferred in vivo model organisms for studying diverse processes like embryogenesis, cell migration, organ formation, behavior, and gene mapping[13]. The one-to two-inch long fish has become crucial as an animal model for mapping genomic functions in developmental disorders and diseases[3]. In addition to easy maintenance and a simple shrimp diet, zebrafish provide large-scale research because they are easy to breed, producing 200-300 eggs/week[12]. They are also useful in detecting gene function because their early stage embryos grow outside the mother at rapid speeds, with major organ formation completely developed in 24 hours[12]. It can sometimes take less than three days for the fish to fully develop and exhibit results, making monitoring mutative effects easier and faster[13]. Furthermore, their transparency in all life stages including egg, larvae, and adult, allows for significant displays of organ development[12]. Unlike the rodent model, where you have to kill and touch the specimen to study them, zebrafish's near transparency allows observation without extensive intrusion[12].

The first time zebrafish were used as a model for human disease was in 1998[12]. The zebrafish mutants were observed for their changed gene, sau (sauternes), which was shown to have a defect in hemoglobin production[12]. The gene encodes for a synthase necessary for hemoglobin biosynthesis. When this gene was turned off, zebrafish developed congenital sideroblastic anemia, a symptom similar to that of humans with defective hemoglobin production[12]. The first transgenic cancer model in zebrafish was for acute lymphoblastic leukemia, the most common type found in childhood leukemia. Scientists generated a T-cell leukemia model by encoding different genes and fusing promoters. Within two months, the injected zebrafish developed tumors in the thymus, spreading to the gills, eye, abdominal organs, and muscle[12]. Many other zebrafish models for leukemia showed sensitivity to the same chemotherapeutic drugs used in human patients. Therefore, zebrafish can and are being used to screen new drug therapeutics[15].

Already zebrafish have successfully been used in studies surrounding unknown genes involved in rare forms of muscular dystrophy, a weakening musculoskeletal system disease that hinders movement[14]. They've also helped scientists better understand the genetic pathways involved in human embryo development and heart physiology[15]. Additionally, drugs developed

Image 9

Genetically modified zebrafish easily show mutation because of their ranslucence[28].



Phenotype 3dpf **Cartilage 4dpf**

from zebrafish research are now being tested in humans for treating skin cancer[15]. By knocking out genes in zebrafish and studying its effects, scientists believe they've found many thousands of potential compounds responsible for heart disease, which are hypothesized to be the next drug for the condition[15]. With zebrafish, researchers can quickly observe the model to see if the compounds truly have an effect on a disease and if the developed drug would be a suitable treatment. As we can see, the zebrafish model has been an effective tool for studying human diseases. My research concentrates on how *complementary* zebrafish are in understanding the neuropathology behind Alzheimer's disease. I will assess the relationship between the human and zebrafish by reviewing the specific proteins hypothesized to cause the disease and seeing if such proteins are also found in the zebrafish.

Literature Review

In an Alzheimer's brain the cells that make up the nervous system, neurons, stop working efficiently and can't communicate with other nerve cells. Specifically, it has been shown that the neurons stop working efficiently because of the misfolding of the beta-amyloid peptide[7]. This leads to amyloid plaques, which are insoluble deposits, or build-up, of the toxic protein peptide[1]. Beta-amyloid is a fragment of protein cut from APP, the amyloid precursor protein[16]. Beta-amyloid begins as a solitary molecule but eventually begins to accumulate with other beta-amyloid pieces[16]. At first the small clusters are relatively harmless because they are still soluble and can travel freely in the brain, but over a long time period without treatment, the small clusters form into the plaques that cause Alzheimer's[17].

The gathered beta-amyloid not only blocks cell-to-cell synapses, but can also quicken

Image 10

Amyloid-Precursor Protein (APP) in a cell splitting into two peptides. Focus on the beta-amyloid peptide and its activity after fragmentation. It splits and then clumps together to form toxic and damaging plaques[28].

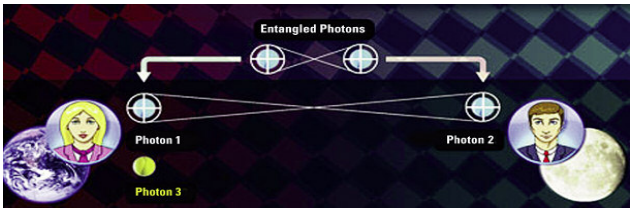


Image 11

The same process in a wider scope of the cell[30].

nerve cell death[16]. The beta-amyloid is a "sticky" chemical and has been shown to strongly bind to receptors on nerve cells that catalyze intercellular processes eroding the synapses with other nerve cells[17]. The idea that there is an accumulation of beta-amyloid in the brain causing cell death is commonly known as the "amyloid hypothesis"[2]. Beta-amyloid plaques in brain tissue may be the primary influence driving AD pathogenesis, or development[17].

A study led by Carla Shatz at Stanford University suggests that beta-amyloid begins the onset of Alzheimer's disease even before the plaques begin to manifest[17]. Using a mouse model, Shatz showed that the mice lacking PirB, a surface protein ordinarily situated close to neural synapses, were resistant to the memory breakdown and synapse loss that follows beta-amyloid accumulation. She concluded that PirB is a high-affinity receptor for beta-amyloid or that beta-amyloid plaques stick to PirB and evoke a medley of biochemical processes to destroy neuron and nerve connections[17]. Although PirB is a mouse specific protein, LilrB2 has been shown to be an analogous protein beta-amyloid receptor in the human brain[17]. Taeho Kim, a postdoctoral scholar in Shatz's lab later compared mice models lacking PirB in their brains with those expressing it. The mice with PirB showed increased activity of a few important proteins, especially an enzyme called cofilin[17]. Later it was shown that cofilin works by breaking down actin, a building-block protein essential to keeping neural synapses intact. In conclusion, beta-amyloid binding to PirB resulted in biochemical changes to cofilin that in turn, started the breakdown of actin[17]. Without actin, there can't be any neural synapse, "boosting cofilin activity busts synapse structural integrity"[17]. Besides its build-up of plaques that block neural connection, beta-amyloid may also be the cause for early cell death by binding to and breaking down proteins integral to cell connection.

Neurofibrillary tangles, thought to be a by-product of beta-amyloid buildup in the brain, are a massive amount of twisted protein threads called tau that collect in the brain's nerve cells[7]. In a healthy brain, tau proteins bind to microtubules, cellular structures that internally support healthy neurons[7]. Tau is essential in keeping the transport system of a cell in its correct

Image 12

Beta-Amyloid clumping together to form amyloid plaques[7].

Image 13

We can see the Alzheimer's tissue has fewer nerve cells and synapses than the healthy brain. Plaques are seen between nerve cells[7].

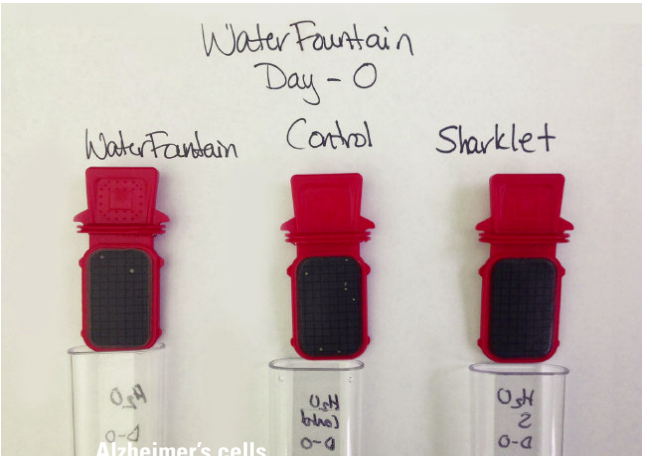
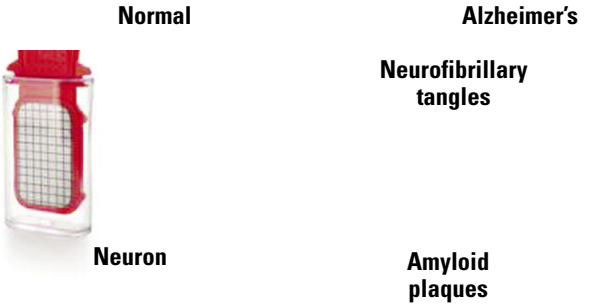


Image 14

Clear neural pathways versus AD brain system cluttered with toxic buildup[7].



parallel strands (something like railroad tracks). In these strands, the organized system can efficiently transport nutrients, cell parts and other key materials[7]. But in AD, the tau protein detaches from the microtubules and collapses into the twisted strands known as neurofibrillary tangles[7]. Without the tau proteins' support, microtubules start to disintegrate and the transport system is no longer parallel. The neuron's internal transport network shuts down as the 'tracks' fall apart and decompose. Without nutrients and other essentials being supplied to the cell, it

eventually dies[7]. Thus, the patient will suffer from the extreme memory loss and dementia that is affiliated with Alzheimer's disease[1].

It still remains unclear how exactly the disease process begins and which proteins treatments should target. But understanding the genetic background that onsets the protein build-up and creates plaques causing Alzheimer's could lead to an important breakthrough in discovering the origin, and thus, sometime in the future, preventative drugs or therapies[1].

Implications of Zebrafish Model for Alzheimer's Disease in Humans

APP is the precursor to beta-amyloid, as explained above: it is the gene that fragments into the beta-amyloid essential to the neuro-degeneration associated with Alzheimer's. The APP gene has two routes for protein expression in the human. The predominant pathway does not generate beta-amyloid, while the other, by a combination of two proteases (β- and -secretase) does[18]. Along with other important proteins in Alzheimer's disease, zebrafish have two homologues, or chromosomes that are similar physically and genetically to another chromosome, of the amyloid precursor protein (APP) with about 70% similarity to the APP found in humans[19]. Zebrafish have *appa* and *appb* genes that are "co-orthologs" of human APP[20]. This means that *appa* and *appb* in zebrafish have evolved from a common ancestral gene by speciation from the human genes fragmented from APP and generally retain similar function. In a study using transgenic zebrafish models, a scientist named Lee, and his coworkers, used the zebrafish *appb* promoter to express GFP, Green Fluorescent Protein. The model showed that *appb* is mainly expressed in sub regions of the brain, spinal cord, and the developing vasculature of the zebrafish embryos[18]. In adult transgenic zebrafish, there was an abundant amount of *appb* in the brain, which shows that the zebrafish *appb* gene can drive GFP expression. This understanding in embryonic development and adult zebrafish is essential for later understanding mutant human APP[18].

Image 15

The breakdown of internal transport system in a cell suffering from Alzheimer's[7].

Tangles are forming **Healthy area**

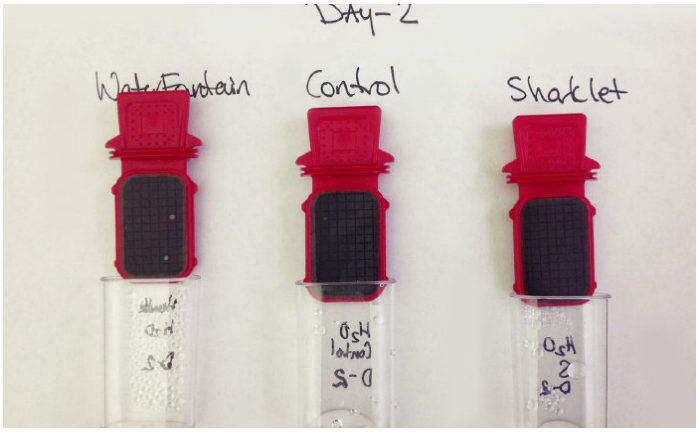


Image 16

Tau protein breaking up a neuron's 'tracks'[31].

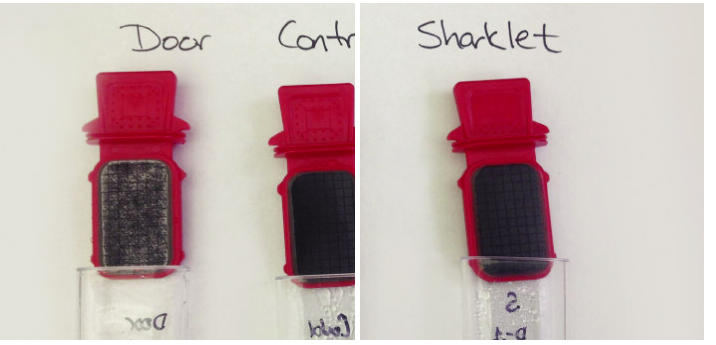
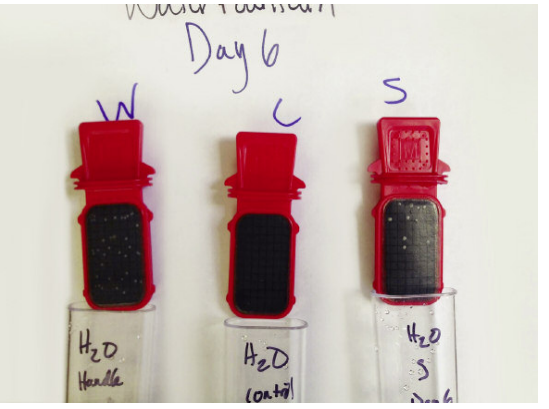


Image 17

Another look at tau protein breaking apart healthy neurons and its changes to the brain[32].



Hands-on Use of a Zebrafish Model

The following is an example of how zebrafish are used to find homologous proteins. For two weeks after Winter Break in junior year I had the opportunity to do an internship at Dr. Richard Kollmar and Dr. Olipriya Dias' lab at SUNY Downstate University. Although this lab was not specifically related to Alzheimer's disease, the procedure is similar to those in labs studying AD. One of the interests of the lab was to find evidence and support earlier claims that certain proteins in the ear contribute to otolith (ear stones that essentially control balance) morphogenesis. When these ear stones malfunction and start to roll out of place, these cause a sense of

Table 1

Genes involved in sporadic and familial AD, the homologous genes in zebrafish and the percentage of protein sequence homology[33].

Human Gene	Zebrafish Gene	Protein Homology
APP	*appa, appb*	70%
PSEN1	*psen1*	74%
PSEN2	*psen2*	74%
Pen2	*Pen2*	74%
Aph1A&B	*Aph1*	62%
Nicastrin	*Nicastrin*	56%
TAU	*mapta/maptb*	Unidentified
APOE	*APOE*	28%

dizziness. It had been found that the Sparc protein is essential in the proper development and fully functioning production of otoliths. The lab had developed a zebrafish model genetically mutated to lack the Sparc protein. The zebrafish lacking the Sparc protein developed otoliths that were smaller, larger, missing, fused, non-functional, extra, and/or exotic, when compared to the wild type. During my time at the lab, I focused on the expression and proper folding of the native Sparc protein. The methods I followed in order to express and study a specific gene in zebrafish are similar to the ones used in other labs using zebrafish as an animal model to study other genes associated with diseases such as Alzheimer's.

The purpose of my experiment was to see if I could manage to express the native Sparc protein and to identify if the functional form was present. The known functional form of the protein is a trimeric form, that is a complex protein formed from three protein monomers. E.coli bacteria were transformed using a plasmid vector that contained the DNA sequence coding for the Sparc protein involved in proper otolith development. In order to maximize protein production, IPTG was added to the bacterial culture. After the appropriate incubation period, I collected the bacterial cells by centrifugation and used sonication to break open the bacteria cells in order to purify my protein of interest. I separated the cell debris from the soluble protein using affinity chromatography. Since the recombinant DNA sequence used for protein expression contained a series of histidine tags, the Sparc protein expressed contained a series of six histidines at one end, used as a tag to purify the protein from the other soluble bacterial proteins by HIS-TAG affinity chromatography. The soluble material was run through the HIS-Tag purification column and the protein of interest was purified and isolated. In order to see if this protein was forming trimers I ran the protein samples in an acrylamide-gel. The acrylamide gels were stained using coomassie dye and the protein bands present were analyzed for the appropriate size (kD) that would form the trimer form of the functional native protein. See image 18.

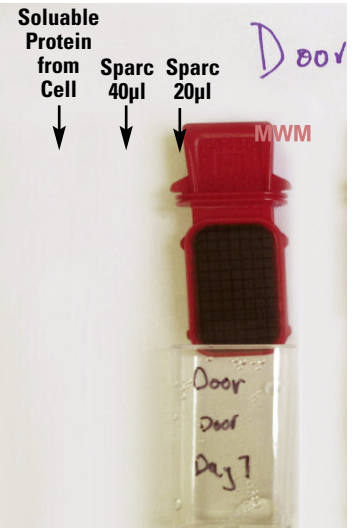
Conclusion

From personal experience and research on how zebrafish are used in the lab, I can conclude that zebrafish are a relevant animal model for studying Alzheimer's disease in the human. Their

orthologous gene expression and function may help scientists develop preventative drugs for Alzheimer's and other neurodegenerative diseases.

Image 18

Sparc protein purification results.



Drawbacks to the Zebrafish Model

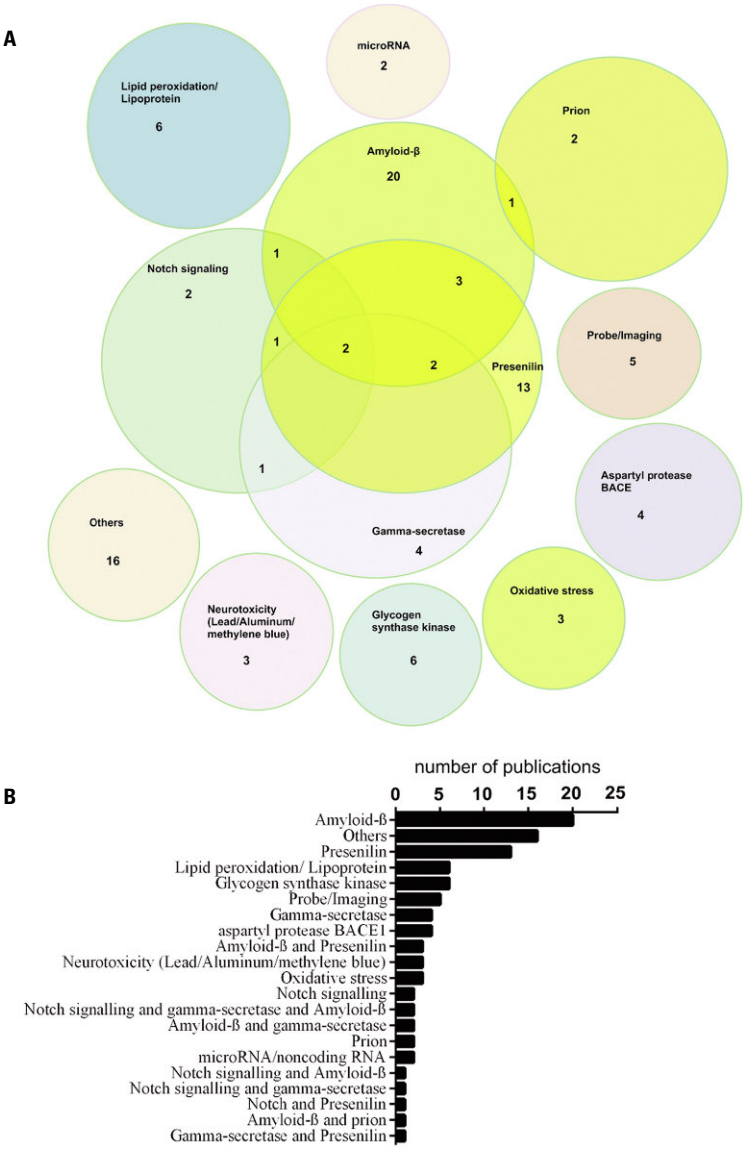
While the zebrafish model has propelled scientists into new and exciting dimensions of understanding Alzheimer's disease, the model also has its drawbacks. There have been some questions concerned with the role of certain structures in the zebrafish, specifically, the frontal cortical and hippocampal structures in learning and memory[21]. The structures cannot be studied in the zebrafish model since these structures don't exist in the zebrafish[20]. These shortcomings are important because the neuron death associated with Alzheimer's disease primarily affects the frontal cortical and hippocampal structures lacking in zebrafish. Although zebrafish have been used in other behavioral research, they cannot accurately display the behavioral changes in Alzheimer's. Lastly, the zebrafish brain has yet to be completely understood and leaves some phenotypic observations underdeveloped[20].

The perils associated with using zebrafish to study neurodegenerative diseases seem to get smaller and smaller as technology develops. At first, there was a lack of genetic tools to accurately assess the zebrafish models. However, in the past few years technology has developed tools such as zinc finger nucleases (allowing efficient gene knock-out), MAZe, and brainbow transgenic technologies, all of which make it possible to see the developmental processes in a vertebrate[21]. More technology will develop with time and the zebrafish model will grow as a helpful tool in understanding neurodegenerative diseases such as Alzheimer's and developing drug therapies.

On the genetic level, duplicated zebrafish genes can have overlapping functions and be disadvantageous for analyzing phenotypes[20]. Duplicated genes come about from unequal crossing-over during meiosis, a specialized process involved in eukaryotic cell reproduction[22]. The homologous chromosomes are misaligned and duplicate. The chance of gene duplication is a matter of sharing repetitive elements between two chromosomes and the consequence includes recombination and reciprocal deletion[22]. Duplicated zebrafish genes make it hard to accurately observe gene function, as the phenotype for that gene will be obscured unless both of the duplicated genes are blocked or knocked out[21]. They can also restrict phenotype and genotype expression to particular cells or tissues[21]. Although these drawbacks exist, the mass of scientific literature on Alzheimer's using zebrafish as a complementary model prove that they do not significantly obstruct our research. A search in the PubMed database using the term "zebrafish + Alzheimer's disease" displayed 119 publications (98 being original research papers)[20].

Image 19

Text mining analysis of the scientific literature to detect papers on the application of zebrafish in Alzheimer's disease research[20].



The Future (Drug Discovery)

Following the amyloid hypothesis, scientists began to look into drugs that reduced amyloid-beta levels in the brain. Testing A, beta-amyloid, lowering drugs in clinical trials on zebrafish was essential in realizing that this sort of treatment should be pursued with caution[20].

In a knockout zebrafish model, *appa* and *abbp* protein translation was blocked by injected morpholinos oligo, an effective gene tool that specifically eliminates the targeted gene[20]. The

results were conclusive. While *appa* inhibition had little effect on the developing zebrafish embryo, *appb* inhibition led to defective cellular movements and a smaller zebrafish body length. Convergent-extension defects as well as defective axon growth and abnormal spinal motor neurons were also observed[23]. The defects were reversed when mRNA coding for human APP was injected into the *appb* deficient embryos[23]. Therefore, from the defective embryos, we can conclude that *appb* loss can cause defective neural development during embryonic development and shouldn't so quickly be excavated from the body with treatment drugs[23]. The beta-amyloid peptide is still poorly understood and scientists initially believed that lowering toxicity levels would keep the neural development in the brain safe and decrease the development of Alzheimer's. But actually, drugs inhibiting beta-amyloid production have shown to lead to significant defects in zebrafish development and should be applied with caution.

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"We look with favor on all forms of learning, but with particular grace we encourage philosophical studies, especially those which by actual experiments attempt either to shape out a new philosophy or to perfect the old."

KING CHARLES

from the 1661
Charter for the formation
of the Royal Academy of Science;
the proceedings of which
are the oldest journal in existence