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The *Journal of Undergraduate Research* (*jur*) is dedicated to providing the student body with intellectual perspectives from various academic disciplines. *jur* serves as a forum for the presentation of original research, thereby encouraging the pursuit of significant scholarly endeavors.

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From the Editors

With every passing year, colleges and universities encourage their students to engage in research as undergraduates with greater intensity. And certainly, this is a good thing – research is educationally transformative, mentally strengthening, and inevitably fosters greater independence on the part of the researcher. But in a way, the formal title of "Undergraduate Research" for such work seems to bifurcate the notion of research into something that one either does or does not participate in. This is especially true when the moniker is used in official programs – say, for example, in the title of this very journal. It can give the impression that only after work is accepted into some program or journal anointed with such a title can it be considered research, and only then does the person become an "undergraduate researcher" who is able to claim all the above benefits.

And that's silly. But this letter is not intended to be a critique of the moniker or programmatizing. Research, regardless of the field, is something much more profound than merely the benefits it confers on the researcher. It is also much more than can be expressed by pithy statements like "the advancement of human knowledge." Research is the legacy of our ancestors' essential need to find answers to the questions that living inevitably confronts us with every day.

Over the millenia, humans have built up immense knowledge on an effectively infinite array of topics, knowledge that fundamentally shapes our world today. But research's real legacy is less that knowledge itself and more countless generations' worth of people determining better ways to construct answers to life's questions. That reflexive need to ascertain truths about nearly everything – say, the behavior of bosons, early Confucian concepts of social order, parental alcoholism's effects on children, surrealist art, appropriate XDR (Extensively Drug Resistant) Tuberculosis treatment regimens – has built structures for making sense of all the world around us, much of which we pick up in our day-to-day lives. But time earnestly spent in college is unique: students' energy is primarily spent on learning and exploring this basic human legacy of research, and in an environment that actively seeks to hone and refine those methods. Whether or not you engage in formal "undergraduate research," college is the time when we take that legacy and make the methods our own.

The image of research-as-ancestral-gift may seem odd, but it is really just an extension of what appears on the previous page in jur's mission statement. In plain English, it says, "We want to bring different thinkers together in hopes that everybody becomes a better thinker." So why have undergraduate research programs that encourage it? Very few places in life invest energy in making you a better thinker; most just require you to be good right away. How much we learn from this legacy does much to determine our ability to shape the world in the future.

Sincerely,

Erika Ilagan Samuel Boyer
Editors-in-Chief

jur

Interview - 103

Perspectives on Research

jur interviews Professor Scott Paauw

Scott Paauw is a visiting Professor of Linguistics at the University of Rochester

jur: Could you fill us in on your educational and professional background?

Paauw: I first discovered the field of linguistics when I as an undergraduate in an exchange program in Malaysia took some courses in linguistics. I was very interested in how language and society interacted - that was back in 1976. From that point, I became a linguistics major. I got a B.A. in linguistics from the University of Michigan in 1979 and then I entered the workforce. Before I finished my undergraduate degree I'd been teaching English in Indonesia; I returned to Indonesia where I taught English for a couple of years, and then I started my own English school. I worked there from 1982 to 2002, running an English school, teaching English, and developing programs to prepare Indonesian students to study at universities in the United States. But I had always wanted to get back into theoretical linguistics, and in 2002 I came back and I did a program in Canada at York University in Toronto, where I got an M.A. in theoretical linguistics. I'm currently working on a Ph.D. in linguistics at the University of Buffalo, and teaching there and here at the same time. So I have 30 years of teaching experience, in both theoretical and applied linguistics.

jur: Could you tell us about any research you're currently involved with and research you've conducted with in the past?

Paauw: The research I've been involved with for the last few years has all related to the dialects of the Malay language, and trying to discover how varieties of the language developed in different geographical locations. And to define the problem itself involves a lot of different questions that have to be answered, in terms of historical dispersal of people throughout the Indonesian islands, the kinds of languages that already existed in areas, contact between different languages, and other historical and linguistic factors that are involved in these things. The first question that I set out to answer was based on something I read in a book that said that Indonesian was basically a Creole language. I set out to investigate that question and discovered it was completely untrue, but that there were contact varieties of Indonesian in various locations of Indonesia that many people considered to be Creole languages, and, through further investigation, I have concluded that none of them are actually Creole languages. A Creole language is a language that has been simplified through contact between two different societies. It's basically a two step process. First, two different groups of people need a language to communicate in; we call that a Pidgin language, which is a simplified variety of one of the languages in the contact situation. Then when people begin using it, speaking it, and teaching it to their children and growing up with it as a native language, we call it a Creole. But my conclusion after looking at the Indonesian situation was that these varieties which have been called Creoles by many observers are not indeed Creoles. The chief evidence for that was that the simplification that was said to have occurred occurs in all varieties of the language throughout the Malay-speaking world. So that was the first question that I set out to examine. And when I got involved in that question, two other problems occurred. One is that there has never been a thorough classification of the varieties of the Malay language (some people might consider these dialects) that are spoken over a vast area, over thousands of miles in area. The language itself has been a language of wider communication for perhaps 2000 years; it's been a lingua franca, a language which is used by groups to communicate between groups that speak different languages, for perhaps 2000 years. And it is dispersed over a wide area, and there's been no classification of the different varieties. So I set about working on a classification of the languages. And in the meantime I discovered that there are varieties spoken even outside of Indonesia. One professor at York University in Toronto, Ian Smith, had worked in Sri Lanka, where he discovered a variety of Malay spoken there, and he collected data on it back in the 70s, but he didn't know what to do with it because he didn't know anything about Malay. So when he met me, he was very happy we were able to work together. We ended up doing quite a bit of research on this variety of Sri Lankan Malay together, and presenting papers on it at various conferences, including the Linguistics Society of America, and publishing a couple of articles about this language variety. I continued to work on that as well. It's a little-known, little-described, little-understood variety of Malay which has arisen because of contact between two very different societies, the

Malay speaking workers who were brought to the Sri Lanka by the colonial administrations of the Dutch and the British and put into contact with speakers of Indian languages, both Sinhala, which is a North Indian language spoken in Sri Lanka, and Tamil, which is a Dravidian (South Indian) language also spoken in Sri Lanka, and these two languages have had a tremendous influence on the structure of the Malay spoken there. These languages have been in contact to the point that the Malay spoken in Sri Lanka is not intelligible in any way with the Malay spoken in Indonesia, although the words are exactly the same. So what they've done is taken the words and put them in a completely alien order. The words look the same, but they have a completely different word order, to the point that even the written language can't be understood. I found that very surprising. It's as if somebody took English and rearranged the words so we couldn't understand what they were saying, they rearranged them so much that we had no idea what was going on. The whole idea of language contact, what happens when two languages meet, is a fascinating one to me, and by its very nature it's interdisciplinary, because we have to look at historical factors, linguistic factors, sociological factors, and all of these interplay in a very interesting way. A lot of my analysis of Sri Lankan Malay has had to do with the socio-historical factors, rather than the linguistic factors, because they've made the language what it is today. And one of the articles that I've published on this topic was co-authored with a historian, B.A. Hussainmiya, because history is just as important as linguistics when we're looking at these questions. Other than that, I'm involved in a few other research questions at the present, involving both Malay and non-Malay languages, other languages of Indonesia, Indonesian, in particular. There's so many questions to be answered, and just a limited amount of time to look at them. That pretty much covers the research I'm doing at present.

jur: What are your future goals with your research?

Paauw: I'd like to collect a lot more data through linguistic field work mainly in Indonesia on varieties of Malay, so I can have a much more definitive typology of the different languages, a much more definitive picture of the variety of linguistic diversity that goes under the banner of the Malay language. The Malay language, by the way, just for a little background, is a major world language. It's spoken as a first or second language by probably close to 250 million people, and it's a national language in Indonesia, the fourth most populous country in the world, Malaysia, Singapore, and Brunei. It's a minority language in Thailand, the Philippines, Sri Lanka and the Netherlands. It's pretty much a language that's unknown to people in this country; even though it is a major world language with a large number of speakers and a large literature in and about the language, it's pretty much a mystery to Americans. Indonesia has been called the best-kept secret in the world, because of various historical and societal factors, but it's an area that's of great interest to me. So, I'd like to eventually come up with a definitive classification of the language and the varieties of the language. There are all these other topics I know that will interest me along the way, but that's sort of where my path is headed right now.

jur: How do you feel that a diverse background in so many different disciplines has enabled you to excel in your research? Does it provide you with a distinct advantage?

Paauw: Absolutely. In linguistics there seems to be a tendency and has been a tendency over the last 30 years or so to focus only on the field. Anything that isn't focused solely on the synchronic analysis of the language (the language as it is now) is somehow regarded as irrelevant. To me it misses the whole beauty of language and the most exciting part of linguistics, which is how cultures interact, how cultures define their language, and how contact between languages, cultures, and societies reshapes the language. The historical factors that play both within one language and between languages are to me the most exciting part of the language. So, I open up my research to many different disciplines when I'm looking at languages. There are more and more people doing the same sort of thing. There are areas of linguistics, such as historical linguistics, which looks at the historical side of things, and socio-linguistics, which looks at the social factors that shape linguistics, which are a part of the field, but are not the most highly regarded parts of the field, because they are not solely interested in theoretical questions. The theoretical questions which have been investigated by linguists in most programs are fascinating questions and they are very important questions and I don't mean to make them sound at all unimportant, but there are other things out there and other disciplines that are important. One of the most interesting theories that has come along in the last ten years or so is a set of theories of human settlement, the idea that mankind evolved in Africa and then went from Africa and settled the world, and that this can be reconstructed. The patterns of settlement can be reconstructed using genetic data, archaeological data, and linguistic data to trace the settlement of the world, the waves of migration that have made the planet what it is today. There has been some fascinating research integrating these different areas by geneticists such as Luigi Luca Cavalli-Sforza. In his book *Genes, Peoples and Languages*, he traces the history of mankind through genetic data, linguistic data, and archaeological data. In my part of the world, there has been some work in this area as well. Cavalli-Sforza has basically mapped the entire world and the genes of the entire world and his work alone is already relevant. There have been historians as well, such as Peter Bellwood from Australia and Leonard Andaya, who have looked at the prehistory of the Indonesian isles, the original settlement patterns, the archaeology, and then worked with linguists to coordinate it with linguistic data so that we have an idea of the settlement patterns of the Indonesian islands dating back three or four thousand years. So the only reason we are able to have these understandings is by combining the research in different disciplines. If an archaeologist only digs up something and doesn't share it with other disciplines, we have no idea how it applies and how it relates to settlement patterns that we can see through genetic data or linguistic data. This can be done only by combining all the different areas and theories of human settlement, such as the theories posited by Jared Diamond. It was something in one of his books that led me to one of my first research questions. He integrates language into everything he does, which I think is fascinating. And he does have a solid understanding of linguistic theories,

a brilliant, brilliant guy, although it was a mistake in one of his books, *The Third Chimpanzee*, that led me to my research. So I have always had an interest in a lot of these areas, especially history, although I have never specifically studied it as an academic topic. However, in my heart, I have always been a historian, and I think it does provide me with an advantage in approaching linguistic problems because I am not tied down by strict adherence to one approach. I can actually say exactly where the error in Diamond's book came from if I trace back. There was a linguist named Robert Hall, who used to be at Cornell for a while and then he was at Texas; he has passed away now. He wrote a wonderful book on Pidgins and Creoles back in the sixties, but there was one line in the book where he said that Indonesian appeared to be a Creole. I don't know where he got the idea, we can't ask him now, but he was listed in the bibliography of Diamond's work. I assumed that was where the error came from.

jur: A lot of your work has to do with the peoples of the Indonesian Archipelago; why does this hold particular interest to you?

Paauw: Well, I spent most of my life in Indonesia. I first went there in 1959 when I was a small child, two years old, and except for two years spent studying in the United States and two years in Canada, it has been my home for the past 35 years. So its incredible linguistic diversity has always been very exciting for me. There are about 300 languages spoken in Indonesia. There is the excitement of watching a relatively new language grow and expand in the language of Indonesia based on Malay, which has become a language of all levels of society, academic study, and every discipline. Watching the language grow and develop has been something I have always enjoyed. And my interest in speaking the languages of Indonesia has made it the area of my primary focus. I have spoken Indonesian languages since I was very small.

jur: What are you doing here at the University of Rochester?

Paauw: At present I am a visiting instructor in linguistic morphology. Morphology is a term that appears in many disciplines, and it is a term that means something different to a zoologist than to a linguist. In linguistic morphology we look at the units that make up words. In the case of English, for example, this parsing consists of affixes, prefixes and suffixes: the Latin suffixes that give high school students so many headaches when they are trying to study for the SAT, and simple verbal inflection in English like the "ing" or the "ed" or the "s" at the ends of words. In English we have a fairly simple system, but many languages have slightly more complex systems, or complex ways of looking at the world. In the class, I have tried to give the students an opportunity to see examples of how other languages break down the world linguistically and to talk about the linguistic theory behind the units that make up words to give a basis for further learning for the students in the course. So, I am here at the U of R for this term and hopefully something will come up again in the future. I certainly have enjoyed my time here. I find the school an outstanding one and I find the students motivated, interested, and a pleasure to teach.

jur: Do you work with undergraduates or supervise independent study?

Paauw: Here at the U of R I'm only working with the students I'm teaching. I also teach at the University of Buffalo, where I have students I work with every semester. I am not directly supervising any independent study, but I do advise students who are looking for ideas and are working on various projects. I also like to be available for students all the time. My students here, I'm sure, will know they can always use me as a resource in the future, even if I'm not teaching here.

jur: What opportunities exist for undergraduates interested in becoming involved in research in your field?

Paauw: There's a tremendous amount of opportunities. If we're considering my field, Indonesian linguistics, which is what I consider it, Indonesia is a country with hundreds of languages, many of which have been little-studied and little-described. For anyone who would like an interesting experience living overseas and doing linguistic field research, there is a tremendous amount of activities in a country that welcomes foreign scientists and academics, and a society where there's a wealth of information that we don't know and can be learned and will help add to the sum of our knowledge. If fifty students came to me and asked where they could work in Indonesia, I could give them fifty different answers, and it would help us understand linguistically, historically, and socially what is going on better. Additionally, there are exchange programs in Indonesia for students, and in other countries in Southeast Asia, which I highly recommend as well, places like Malaysia, Singapore, and the Philippines, which all have related languages spoken in them. My areas of interest are ones that definitely would welcome undergraduates who are interested in them. Short of leaving the country, it's pretty hard. At the University of Rochester, no Indonesian languages are taught. The closest place that I know teaches Indonesian languages is Cornell. There are about seven or eight programs in the United States, which is pretty sad for one of the largest languages in the world, for the fourth most populous country in the world; there's maybe seven or eight out of 2700 schools in the United States where it's taught - Cornell, Yale, Berkeley, Hawaii, Northern Illinois for some reason, and the University of Washington.

jur: In your opinion, how important is interdisciplinary work to your field and in general?

Paauw: I'm a strong advocate of interdisciplinary work. I think that when a discipline has blinders on it, it really can't move forward, it's not really adding to human knowledge. It's when you can expand your horizons, when you can see what other people have done. When you look at Jared Diamond's work, for example, I mean, he's a biologist! He first got into this whole thing when he was studying the development of plant and animal life in New Guinea. That led him to studying how this happened, and then he started studying the history of human societies, and that meant that he had to have historical information, linguistic information, agricultural information,

sociological, genetics, everything: it all came in. There are linguists who are working on this sort of thing. There is a fellow at Stanford named Merritt Ruhlen who is trying to bring everything back to the mother language that all human languages descended from, and finding patterns of the descent, drawing a family tree of all the languages of the world. And he works correlating his findings with genetic data, because the genetic data is sort of the family tree of the world - we're all related; despite what some wacko might believe, everyone is related, and we all started in Africa, and it can be traced. There's only one human race; it's just different ethnic groups among it.

Role of Immune Responses in Pathogenesis of nontypeable Haemophilus influenzae in Acute Otitis Media in Young Children

Bethany Kopin, 2006

Advised by Michael Pichichero, M.D.

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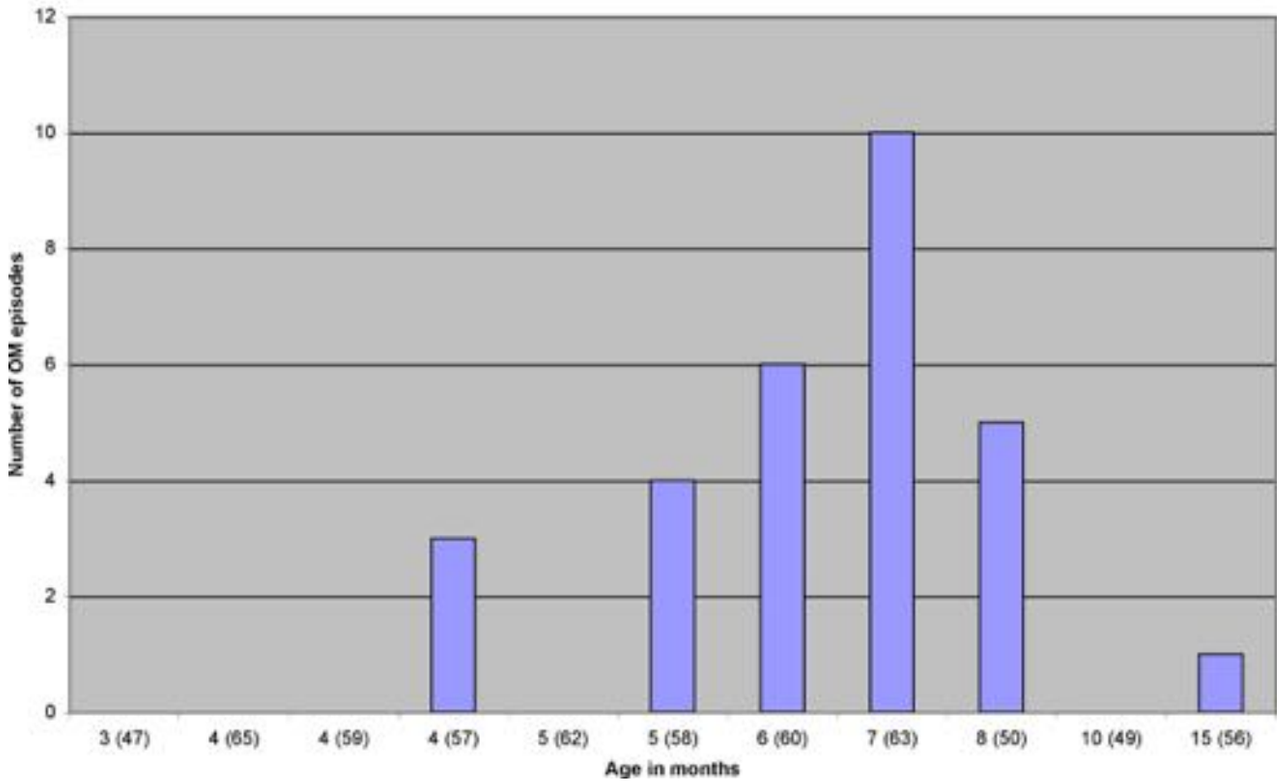
Accounting for approximately one out of three pediatric visits for children two years of age and under, acute otitis media (AOM) is one of the most common illnesses in young children.[1] Most children have their first encounter with AOM before their first birthday. This illness has the potential to have devastating effects on a child's speech, academic achievement, and social development.[2] Several factors that identify a child at risk for recurrent disease include having the first episode of otitis media early in life, having other family members with the recurrent disease, enrollment in day care, and bottle feeding.[2] In order to minimize the adverse effects of this disease, it is necessary to identify characteristics that may predispose a child to serious AOM infection.

The overall cause of these infections is a bacterium commonly known as *nontypeable Haemophilus influenzae* (NTHI). This gram negative bacterium colonizes the nasopharynx of children, and then spreads through the Eustachian tube (ET) to the middle ear where an inflammatory response occurs. The success of this organism as a colonizer and pathogen is due to its lack of reliance on any single method of attachment and its ability to respond rapidly to host defense mechanisms by antigenic variation of proteins and enzymes. Another unique characteristic that separates this bacterium from the closely related *H. influenza type b* is that NTHI lacks a polysaccharide capsule. Since the current vaccine against the *H. influenzae type b* strain is designed to target the recognized polysaccharide

Fig. 1 Number of Episodes of AOM as a Function of Age



capsule, it is ineffective against the non-capsulated NTHI.[3]

One possible method of intervention is the development of a vaccine that targets specific outer membrane proteins (OMP's). In other studies, the surface protein P6, among others, has shown particular promise for this purpose. P6 is a highly conserved OMP found in all strains of NTHI and is expressed on the surface of NTHI throughout all stages of infection. Therefore, the use of P6 as a vaccine component appears to be the most effective way to inoculate otitis-prone children against NTHI.[1]

Consequently, one proposed mode of protection against this bacterium is the possibility of an immunologic antibody response to P6, thought to be initially generated by the ET, which is the proposed mucosal effector site of this pathogen. This immuno-competent organ can be activated with appropriate antigenic stimulation and in turn bring about the proliferation of effector molecules known as immunoglobulins.[4] Throughout various stages of infection, the dominant immunoglobulin class of antibody produced in humans against the surface protein P6 is IgG.[3]

Otitis-prone children experience an immunologic abnormality that causes them to experience recurrent infections. Several lines of evidence suggest that antibody levels to P6 are higher in non-otitis-prone children compared to otitis-prone children, implying that antibodies to P6 are relatively protective against otitis media. Moreover, the levels of IgG anti-NTHI antibody decline to the lowest level at six months of age and remain relatively low until two years of age. This range corresponds to the statistical values that show the greatest incidence of otitis media in the first few years of life. This insufficient quantity of IgG in otitis-prone children may account for the failure to recognize P6 as a specific immunogen and for recurrent infections,[5] suggesting that otitis-prone children may not respond adequately to a vaccine containing P6.

Another potential method of protection from NTHI is the body's use of bactericidal activity, which describes the body's natural ability to prevent infection by inhibiting the growth or action of microorganisms by the use of specialized immune system components such as complement. This study utilizes these basic body components to further analyze the overall goal of determining a mechanism of intervention against NTHI.

The purpose of this study is to determine specific factors that play a role in immunologic protection against NTHI. These hypothetical factors include antibody against whole cell bacteria, antibody against the surface protein P6, and high levels of bactericidal activity against this organism. The sera of both non-otitis prone and otitis-prone pediatric patients were used to evaluate these aspects of host immunity. Throughout the course of this experiment, various methods and assays, such as ELISAs (NTHI whole-cell, P6), and measurement of bactericidal activity using acute and convalescent phase serum from pediatric subjects with varying otitis media experience, were employed. One of the overall goals of this experiment is to play a role in bringing the current medical community one step closer to the final development of an effective vaccine against NTHI.

Methods

Collection of sera

The samples used were part of an existing collection of sera and matched organisms belonging to Dr. Michael Pichicero. Bacteria were isolated from middle ear fluids of children with AOM during acute phase (day 1) and convalescent phase (day 30) of infection, along with matched sera. At the time of sample collection, a blood draw and tympanocentesis (removal of fluid from behind the eardrum) were performed in compliance with current human subject research regulations. Each subject was given a unique identification that remained confidential.

Whole Cell Bacteria ELISA Assay

The Enzyme-linked-immunosorbant-assay (ELISA) assay was used to determine serum (acute and convalescent phase) antibody response to matched organisms isolated during acute phase of disease. Organisms were grown on chocolate agar (BBL Laboratories, MD) at 37° C for 18 hours in CO_2 to ensure viability and purity. Colonies were then collected and placed in 10mL of phosphate buffered saline (PBS) until an optical density of 0.5 (OD_{600}) was reached. Next, 100µl of bacterial suspension and 100µl of coating buffer (Kirkegaard & Perry Laboratories (KPL), Gaithersburg MD) were placed into 96-well plates. The plate was incubated at room temperature for one hour. After incubation, contents were tapped out and 200µl of bovine serum albumin working stock (BSA 1:1000 Dilution) (KPL, Gaithersburg MD) was added to each well. Incubation was carried out again for fifteen minutes. Contents were again tapped out after incubation and 100µl of human sera was added to the first 2 columns of the plate. With the exception of the last column (column 12), a ten fold serial dilution was then performed across the remaining columns of the plates. Column 12 was left as a negative control, and therefore no serum was added. After an hour of incubation, wells were tapped out and washed using a washing buffer working stock. 200µl of this solution was added to each well, and contents were tapped out of each well into a waste receptacle for a total of three times. 100µl of diluted secondary anti human IgG of goat origin (alkaline phosphatase conjugate [Biosource Laboratories, Camarillo, CA]) was added to each well. The plate was then incubated for one hour at room temperature. After incubation, the washing step was repeated. An ELISA kit (KPL) was then employed, and prepared according to manufacturer's instructions. Plates were then developed using P-nitrophenylphosphate substrate, also from KPL. After development, absorbance was read on a Dynatech ELISA reader at a wavelength of 405 nm. Absorbance values were divided by controls to yield an absorbance of response index value.

P6 assay

ELISA assays were also performed to determine the degree of patient sera immune response elicited against the OMP known as P6. This assay was performed identically to the Bacterial Whole Cell ELISA as described above with the exception of using sera at a consistent dilution of 1:1000. The purified P6 antigen used in this aspect of the experiment was a gift from Dr. Timothy Murphy, SUNY Buffalo, Buffalo, NY .

Fig. 2 Immune Response to NTHI in Sera from AOM Patients as a Function of Age

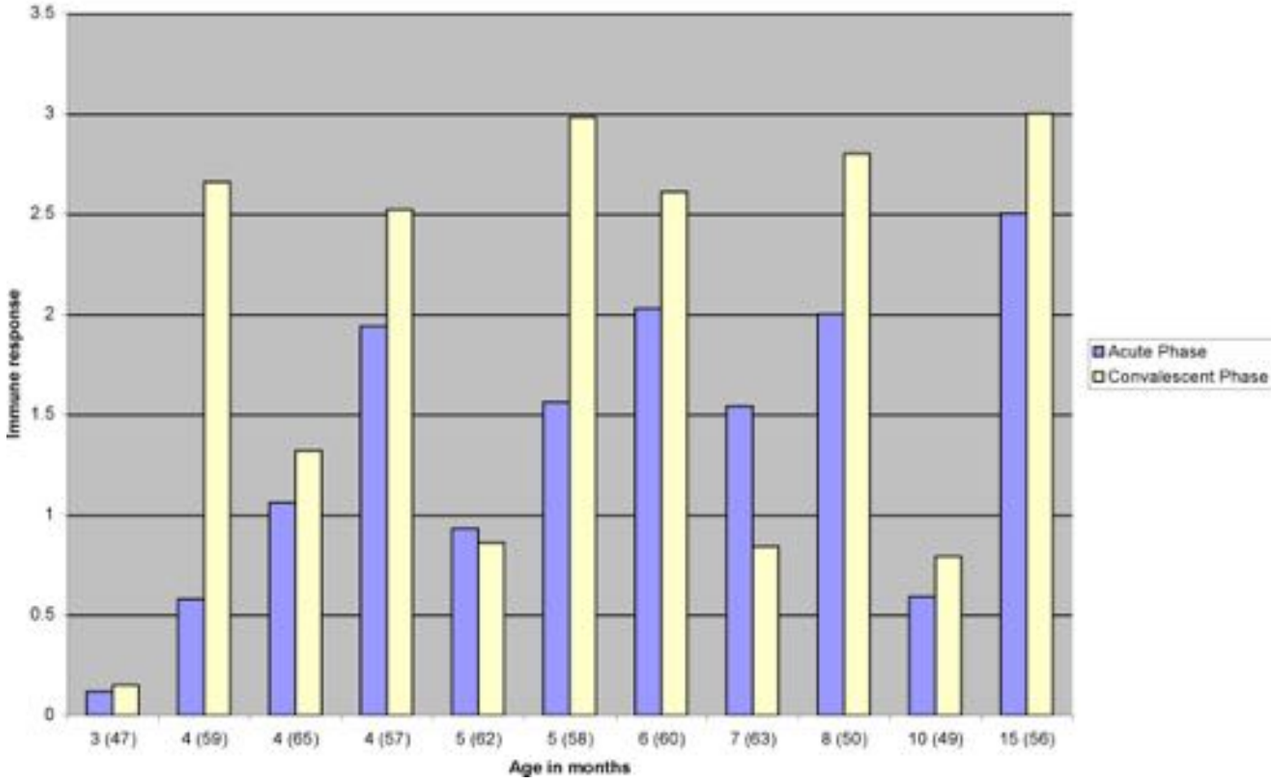
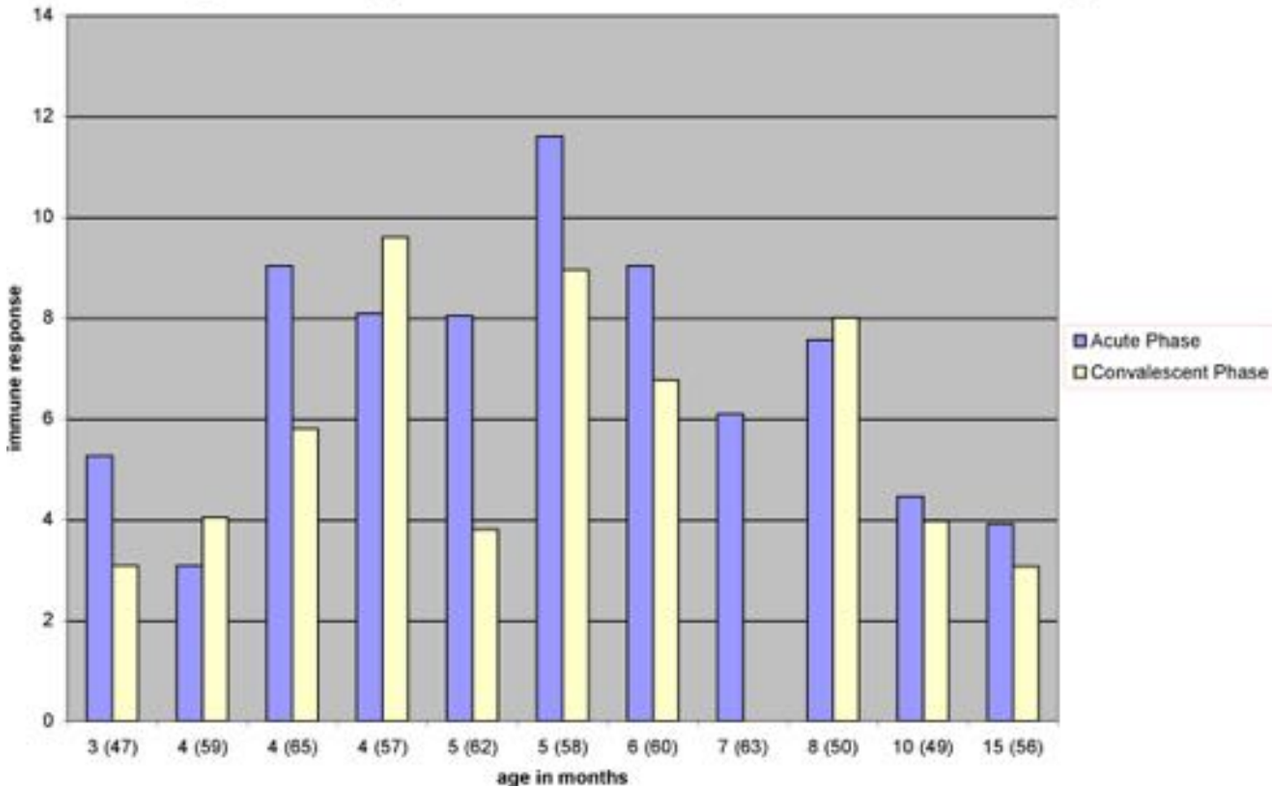


Fig. 3 Immune Response to P6 in Sera from Patients with AOM as a Function of Age



Bactericidal assay

The serum bactericidal assay is a functional measure of the ability of antibodies in conjunction with complement to kill bacteria and is consistent to measure functional antibodies in vitro. This method was engaged in this study for that purpose.

NTHI isolates were grown in BHI (brain heart infusion) broth (BBL) containing X factor (lysed huRBC), and V

factor (NAD). The bacteria were then incubated at 37° C with shaking until an OD_{490} of 0.8 (~ $4x10^9$ CFU/ml) was reached. The log phase bacteria were suspended to 2 x 10^4 CFU/ml in 1 x PCMA buffer (PBS + $CaCl_2$ + $MgCl_2$ + 0.1% BSA). Precolostral calf serum was used as a complement source and was diluted to 20% in the bacteria - 1x PCMA mix. Immediately after dilution, the bacteria and complement mix were used in the assay.

The bactericidal assay was performed in a 96 well RB sterile plate. Test sera and control sera were serially diluted in 1x

Fig. 4 Bactericidal Activity Against NTHI from Sera of Patients with AOM as a Function of Age

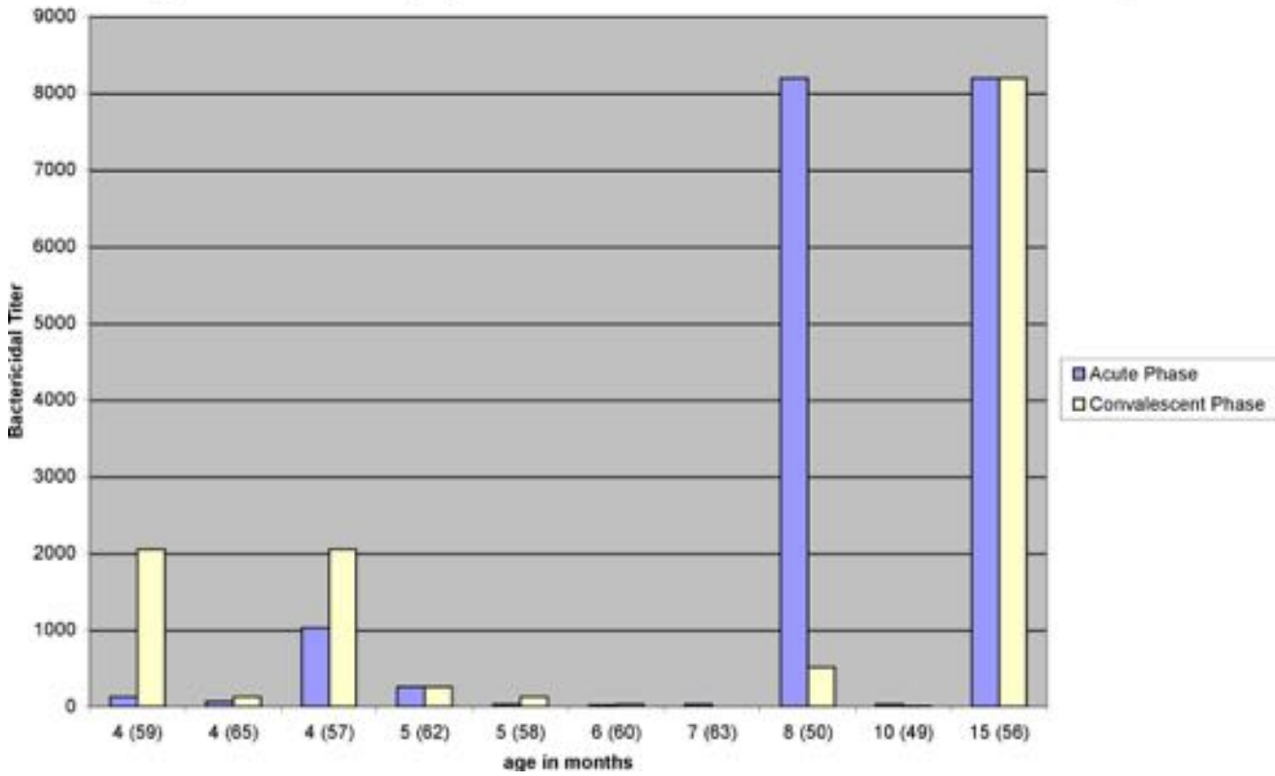


Fig. 5 Immune Response to NTHI in Sera from Patient with AOM as a Function of OM Index

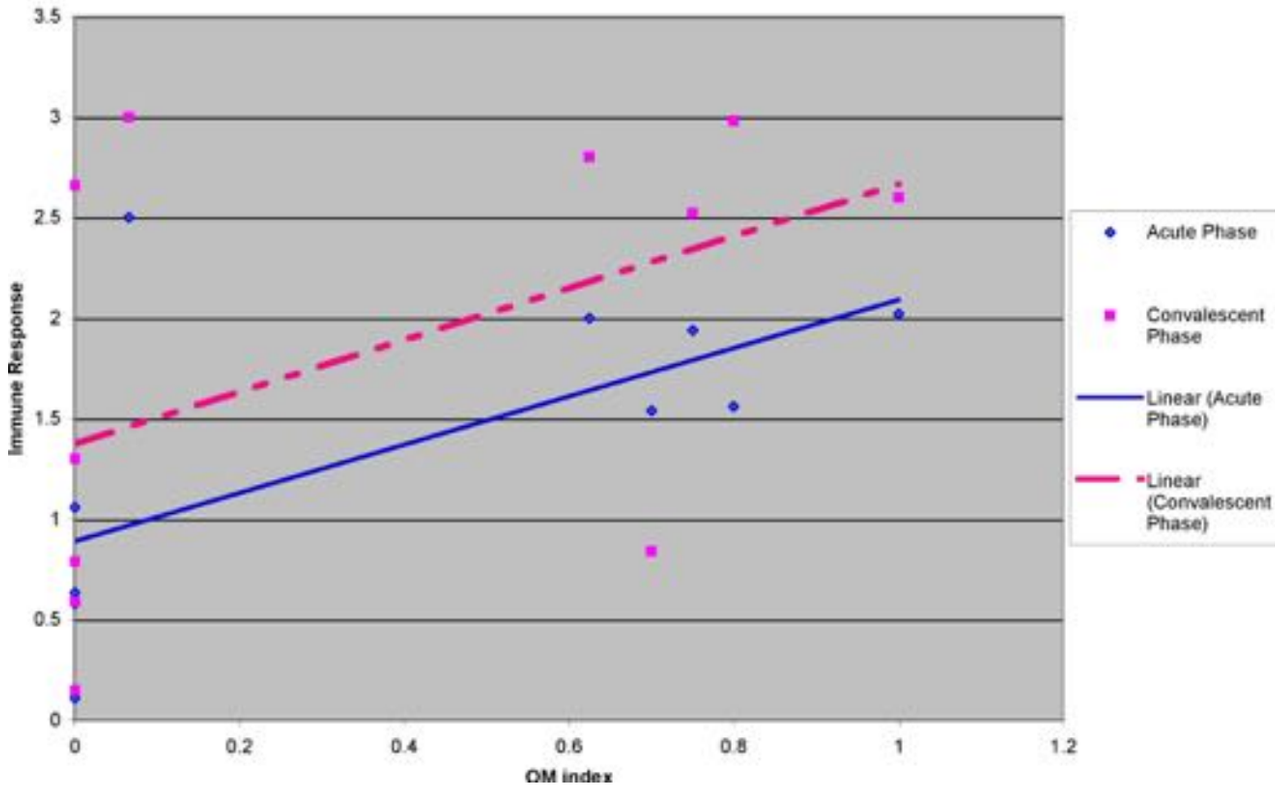
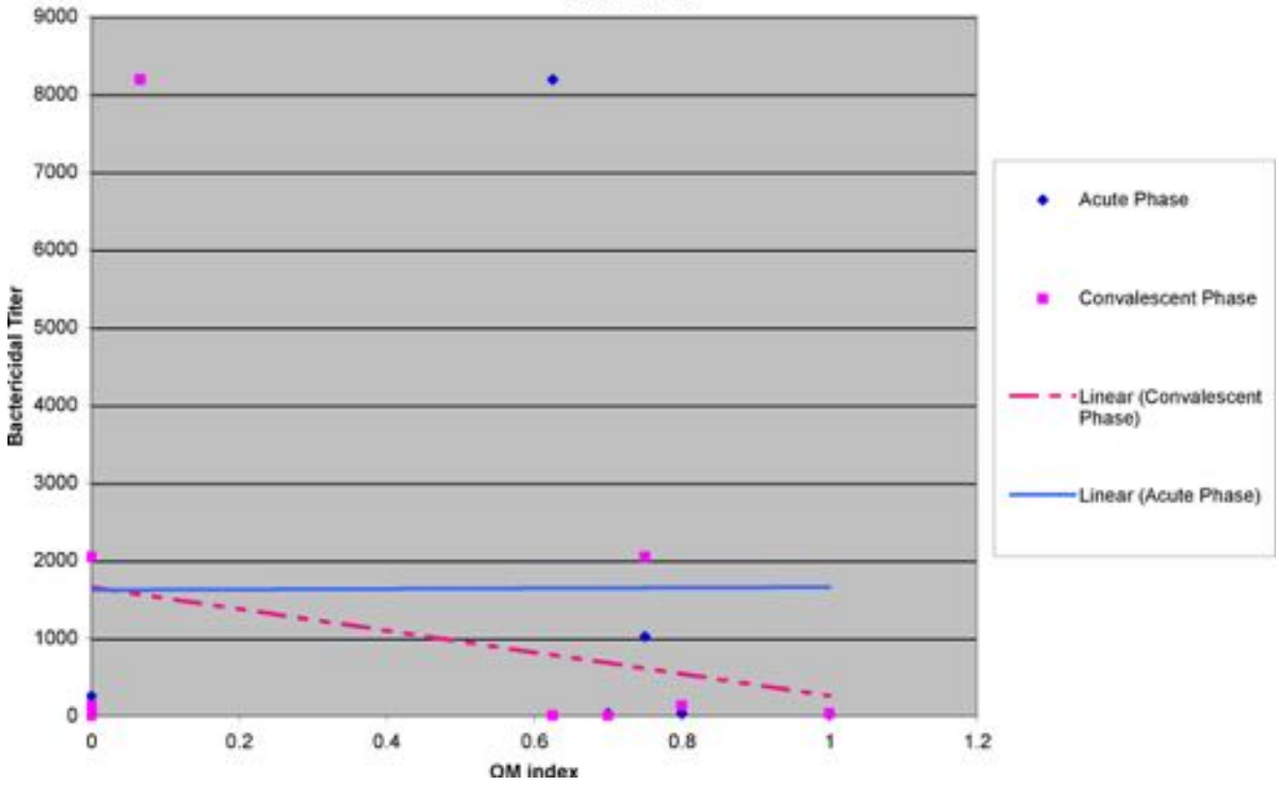


Fig. 7 Bactericidal activity against NTHI in Serum from Patients with AOM as a Function of AOM Index



PCMA buffer. Bacteria and complement were added to the wells containing diluted sample. Bacteria + complement alone and bacteria alone were run as controls in parallel with the samples. After incubation, an aliquot from each culture well was plated onto chocolate agar plates (BBL). The plates were then incubated overnight. Bacterial colonies were enumerated and bactericidal titers were expressed as percent killing versus the negative control (bacteria + complement without antiserum).

Results

There appear three groupings of results that allow for easy analysis of the collected data. Organizing the data in this manner facilitates clear understanding of the overall findings. These three groups consist of the immune response in comparison to age, otitis media index (OMI), and phase of infection. The OMI is defined and discussed in its relevant section below.

Group 1

Throughout the course of this experiment there were several underlying factors that contributed a great deal to the overall results and findings. Two of these factors were the subject's age and the subject's number of previous NTHI episodes. The patients range in age from 3-15 months and have different immunological histories and backgrounds. The progression of AOM can be divided into two major phases: An acute and a convalescent phase. The acute phase corresponds to the time period immediately following initial infection, while the convalescent phase corresponds to the time period 30 days after the initial point of infection. As seen in Figure 1, the patients with the highest number of previous NTHI episodes (otitis prone) are those in the age category of 5-8 months. This category correlates with the time period when IgG production is at its lowest point. Other subjects that are outside the 5-8 months range have fewer number of episodes, and are therefore non-otitis prone. This all-time low production is obviously a major contribution to the high number of OM episodes seen at this point.

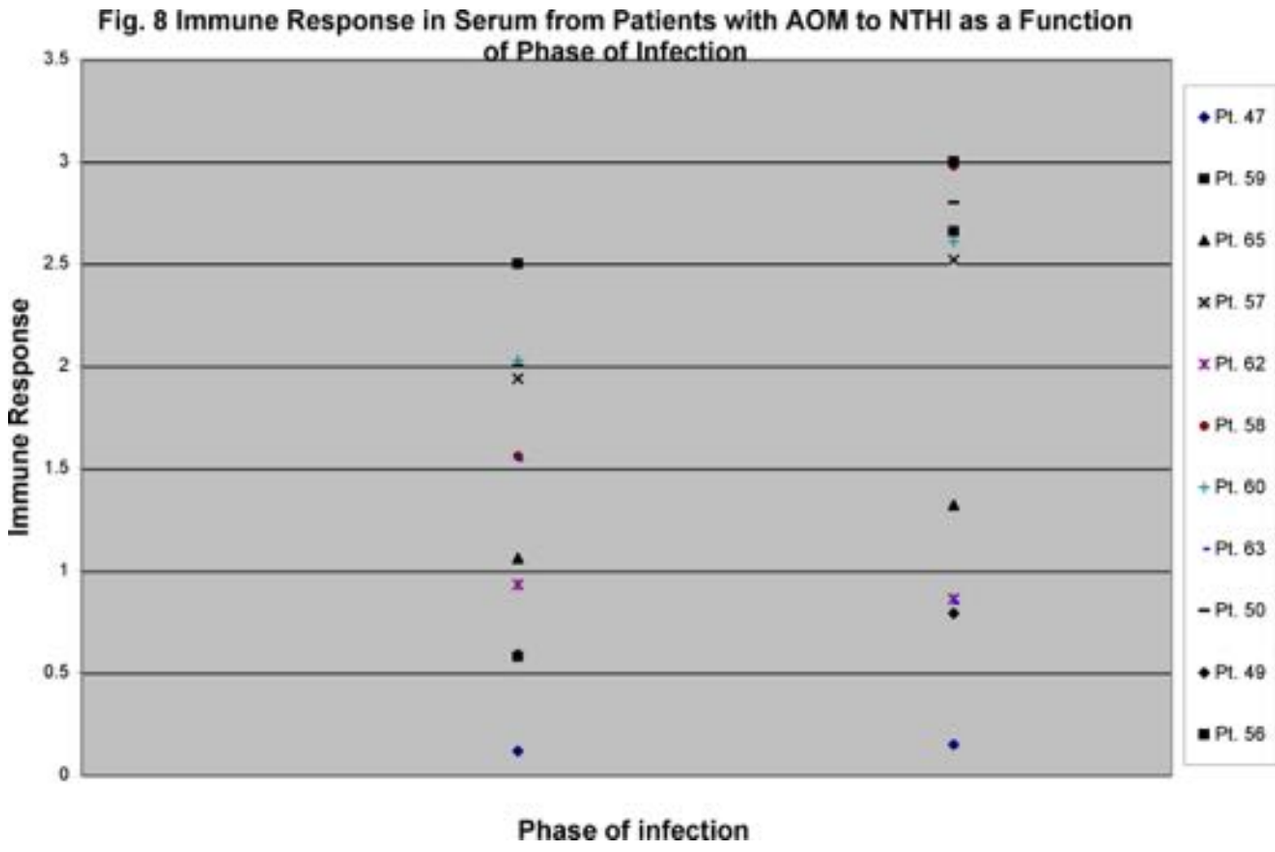
Figure 2 also reiterates low IgG production between the ages of 5-8 months, showing the acute and convalescent phase bactericidal titer versus age in months. In this case the term titer refers to the reciprocal of the last dilution of a titration giving a measurable effect. Bactericidal activity is found to be weakest once again when IgG production is lowest. Moreover, this illustration also shows the ability to increase immune response and antibody production with age: bactericidal activity increases with age and maturity.

Figures 3 and 4 illustrate the difference between the immune responses mounted against whole bacteria versus P6. Comparing the immune response values obtained, there is a relatively weak acute response and a stronger convalescent response to whole cell bacteria. Inversely, there is a stronger acute response and a relatively weak convalescent response to P6. Collectively, this data shows the overall difficulty to retain memory cells against the antigen P6.

Group 2

The OM index is defined as the number of previous OM episodes divided by the age in months. This factor allows us to consider the subject's age in relation to the prior number of episodes as a single variable. Overall, the lower the OM index, the more immunologically fit the individual.

There is an upward incline in the immune response as



OM index increases, as illustrated in Figure 5, implying an actual higher immune response in subjects who also have a relatively high OM index. Also, the difference in height between the acute and convalescent phases indicates successful immunological memory against whole cell NTHI.

The lower a patient's OM index, the higher the immune response generated, as represented by the downward shift in slope as the OM index increases. Figure 6 shows the OM index in relation to the immune response elicited against the surface protein P6. In this case the convalescent phase response is somewhat lower than that seen in the immune response to whole bacteria. These results most likely suggest that the immune system does not mount as strong of an immune response post-infection, indicating little or no immunological memory retention.

Children with low OM indexes mount a better immune response against the purified antigen P6 than against the whole cell bacteria, as shown in Figures 5 and 6. Inversely, subjects with a higher OM index seem to be better at mounting an immune response against whole cell bacteria than to purified P6.

The following various figures show the multiple relationships held between OM index and immunity against NTHI. For instance, Figure 7 illustrates the OM index in comparison to the bactericidal activity against NTHI. This figure differs from Figure 6 in that there is a significantly lower immune response mounted in the convalescent phase in comparison to the acute phase, implying that little immunological memory was formed against the pathogen. The graph also shows that with a higher OM index there is a lower convalescent immune response. This illustrates that otitis-prone subjects are less efficient in mounting a convalescent immune response.

Group 3

The third grouping allows for careful analysis of the trend seen between phase of infection and immune response. Overall, it demonstrates the dynamics seen between the microbe's pathogenesis and the reaction of the body's immune system. For example, Figures 8-10 comparatively examine the relationship between phase of infection and the average immune response mounted by the patients. Figure 8 shows each phase of the immune response elicited against whole cell NTHI. As seen from the graph, there is a significant difference in immune response between the acute and convalescent phase of infection. In this case the immune response in the convalescent phase is considerably higher than in the acute phase, implying successful immunological memory and more probable chance of clearing the pathogen. Figure 9 differs from Figure 8 in that there is almost no immunological memory retained against the surface antigen P6. Figure 10 depicts the immune response between the acute and convalescent phases to be relatively similar. These results indicate that there is very little memory gained between these two distinct phases of infection with regard to bactericidal activity.

Discussion

Experiments were performed in order to determine the relative immune response elicited against NTHI whole bacteria in otitis-prone versus non-otitis-prone subjects during acute and convalescent phases of infection. The acute phase corresponds to the time period immediately following initial infection, while the convalescent phase corresponds to the time period 30

days after the initial point of infection. The results obtained indicate a general increase in immunity between the acute and convalescent phase of infection, indicating a memory response generated after infection.

We also performed assays in order to find the average immune response elicited against the NTHI OMP known

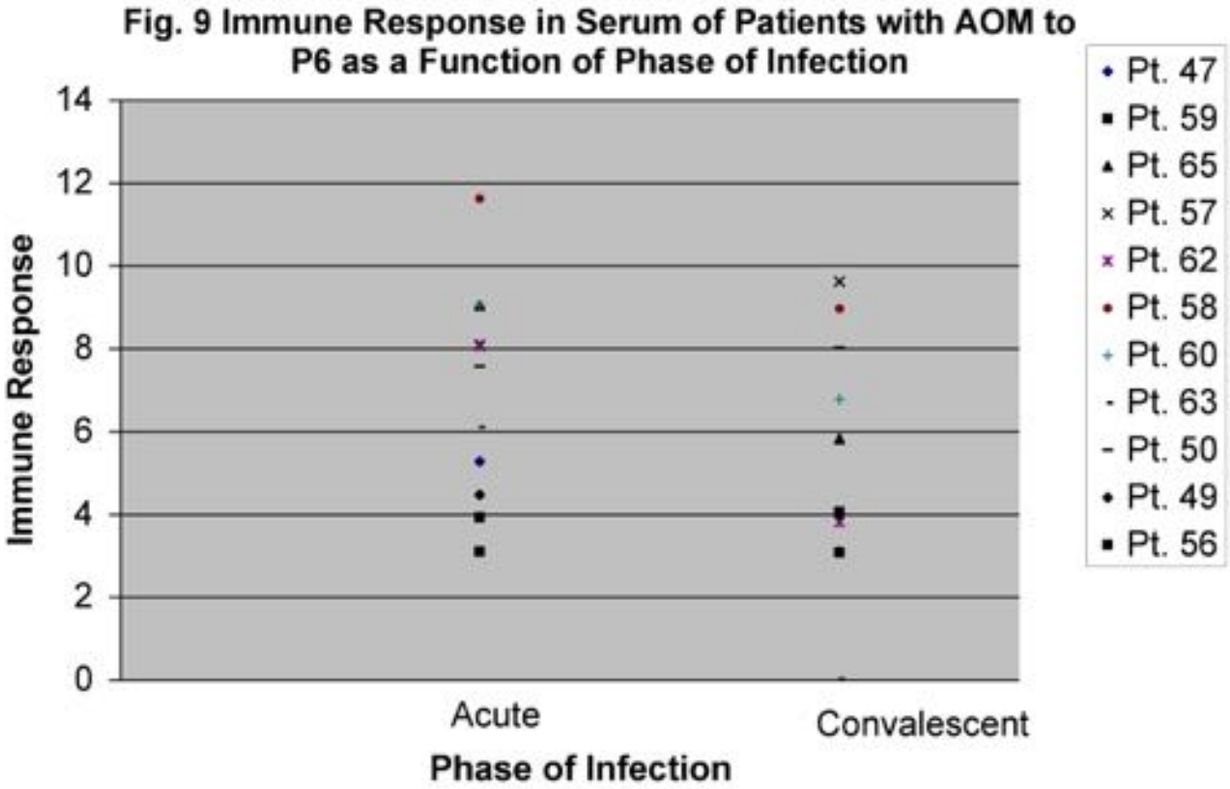


Fig. 9 Immune Response in Serum of Patients with AOM to P6 as a Function of Phase of Infection

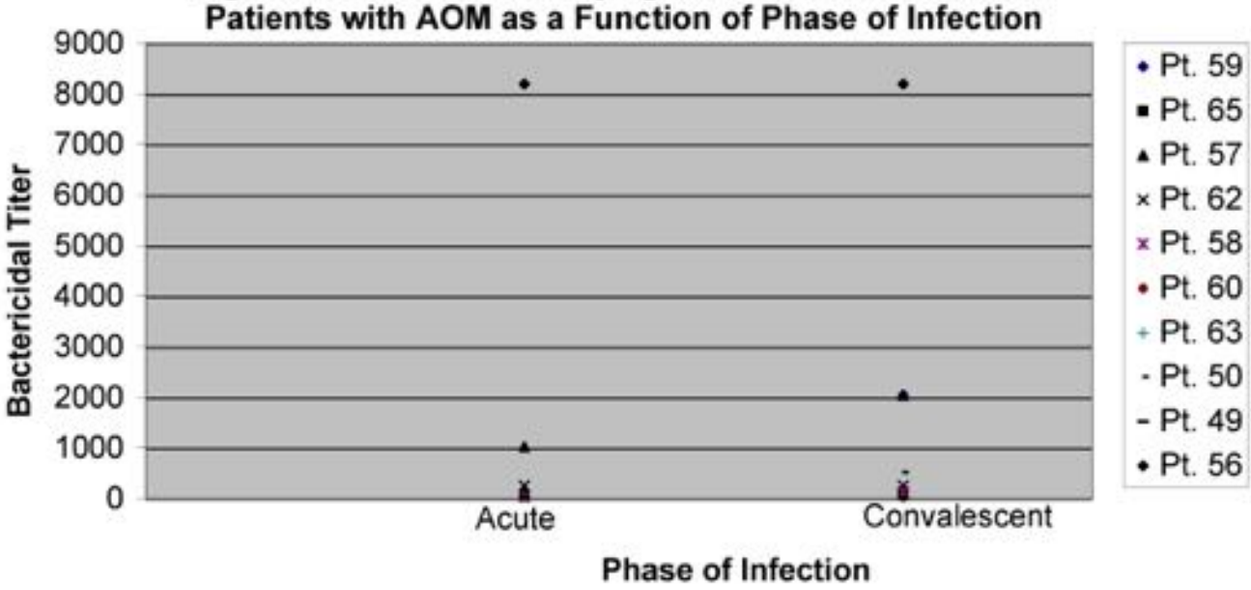


Fig. 10 Bactericidal activity against NTHI from Serum of Patients with AOM as a Function of Phase of Infection

as P6. The acute and convalescent phases were compared to one another to find the degree of immunologic memory. In addition, the patient's vulnerability to this pathogen was taken into consideration by noting his or her number of previous episodes. The results obtained indicate that, in most cases, there is little or no immunologic memory generated after the 30 day period, and this memory differs a great deal from the memory formed against NTHI whole cell bacteria. The degree of response obtained from the acute phase of infection is virtually equal to that of the convalescent phase of infection, implying almost no immunologic memory to the OMP P6.

Finally, bactericidal assays were performed to measure the functional ability of antibodies along with complement to kill bacteria. As with the other types of experiments, the acute and convalescent phases were analyzed to find the underlying difference between the immune response of otitis-prone subjects versus non-otitis-prone subjects. The results illustrate the vulnerability of patients between the ages of 5 and 8 months. The bactericidal activities at these points were at an overall low, reflecting the known low production of IgG during this time period. This figure also shows the ability to increase immune response and antibody production with age.

Non-otitis-prone patients are less efficient than otitis-prone individuals in mounting an immune response against whole cell NTHI, but are more efficient in mounting an immune response against purified P6. Although there is a relatively strong immune response mounted against P6 in the acute phase by non-otitis-prone individuals, there is even less of an immune response mounted in the convalescent phase, indicating that there is little or no memory gained upon the primary infection. These observations indicate that the antibodies that recognize the P6 on the pathogen are not protective, and do not explain the non-otitis-prone patient's intrinsic ability to fight off NTHI infection.

Collectively, the findings from these studies offer something new to the scientific and medical community. A majority of other investigators, such as Dr. Howard Faden, have conducted similar experiments and found P6 to only be a hypothetical vaccine target. The findings here take Faden's work to the next level and show that P6 is not a protective antigen in the pathogenesis of *nontypeable Haemophilus influenzae* in acute otitis media in young children.

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Computers Composing Music: An Artistic Utilization of Hidden Markov Models for Music Composition

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Natural systems are the source of inspiration for the human tendency to pursue creative endeavors. Music composition is a language for human expression and can therefore be utilized in conveying the expressive capabilities of other systems. Using a Hidden Markov Model (HMM) learning system, a computer can be taught to create music that is coherent and aesthetically sufficient, given the correct tools.

> *"A Hidden Markov Model (HMM) is a statistical model where the system being modeled is assumed to be a Markov process [a finite, probabilistic system with state relationships] with unknown parameters, and the challenge is to determine the hidden parameters, from the observable parameters, based on this assumption. The extracted model parameters can then be used to perform further analysis, for example, for pattern recognition applications.*
>
> *In a regular Markov model, the state is directly visible to the observer, and therefore the state transition probabilities are the only parameters. A hidden Markov model adds outputs: each state has a probability distribution over the possible output tokens. Therefore, looking at a sequence of tokens generated by an HMM does not directly indicate the sequence of states."* [1]

Such models have applications to speech recognition software, music transcription, and analysis of incomplete data because of this ability to approximate, as will be further described below. It is this capability of "fuzzy" pattern recognition that inspires the use of HMM's for this project.

The tools selected for this project include: twenty-two years of sun spot data as the natural system from which to creatively draw; a compositional framework for structure, pitch, dynamics, and rhythm to facilitate a human understanding of the system's expressiveness; the jMusic[2], open source, music composition software; and an HMM learning system[3] with implementations of the Forward-Backward, Viterbi, and Baum-Welch algorithms. Details of these tools shall be discussed later. In composing a final piece of music, the attempt was made to impose as few creative restrictions on the system as possible. Through these tools, every aspect of the composition's generation can be repeated. In this way the robust analytical capabilities of the system are displayed via the piece and its generative procedures, thereby displaying an artificial intelligence's potential for music composition and perhaps larger creative projects.

Artistic Motivations

The artistic bases of this project are as follows:

•Nature has discernable patterns within its manifestations

•Patterns are interpreted on a basis of past experience

•Music composition is driven by a need to express relationships

A clear and empirical observation is that natural systems exhibit certain repetitive and cyclic properties. Human beings interact with their environment on a constant basis, and one of the greatest skills we exhibit is the ability to recognize and learn from the patterns that occur within that environment. It is particularly interesting that we seem to be able to observe a pattern within nature and then apply the observations about that series of relationships to other, seemingly unrelated tasks.

Engineering, mathematics, psychology, and any science - martial arts, painting, music composition, and any artistic endeavor - appear to be exhibitions of the human capacity to interpret nature. Inquiries into the foundation of this ability are at the heart of some of the greatest pursuits of philosophical study. What is already apparent, however, is that humans utilize past experience to interpret newly recognized patterns and to build on their base of knowledge.

It is also interesting to observe the creative urge of the human condition. The need to express thoughts in some medium, whether it is through engineering or music composition, is a universal trait. One artistic assumption that was made during this project is that this human creative urge stems from a natural desire to express our interpretations of the patterns and relationships that we have recognized in nature. This does not appear to be an inappropriate leap in logic, because the fuel for creativity must come from nature in some capacity. Without learned concepts and patterns, we would have no basis for creation.

Therefore, it seems possible to imbue an artificial system with the tools necessary to execute a similar process of creation through the medium of music composition; utilizing the proper learning tools (Hidden Markov Models) and with a person to guide the creation and transmission of the product to listeners (jMusic and the composer).

Tools

Two main software resources were used in the course of

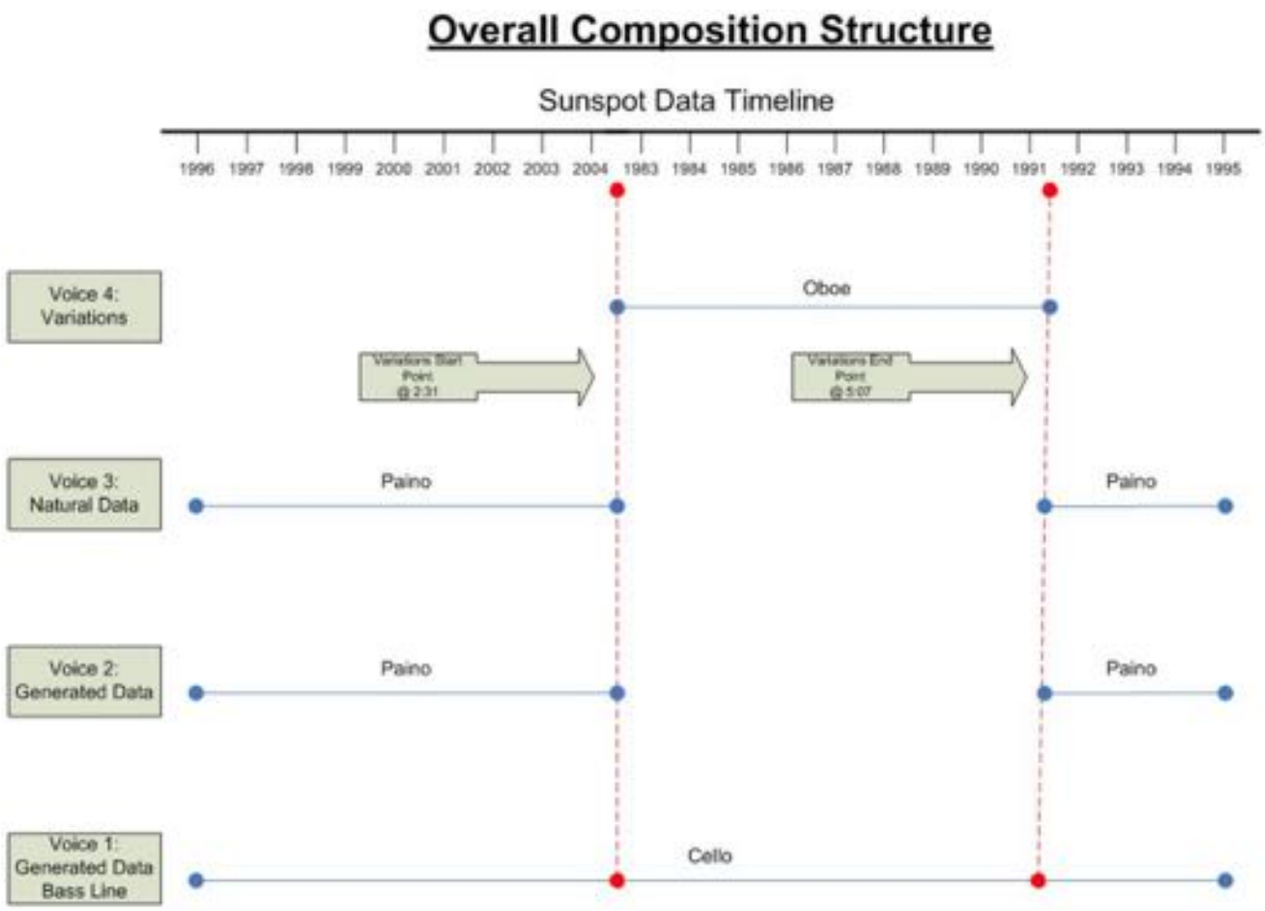


Diagram A: Compositional Structure Flow Chart
Represents the musical arrangement of the piece with respect to the sun spot data's year, the particular voice, and the instrument utilized to articulate that voice.

this project. The first is a Hidden Markov Model (HMM) software implementation of the Forward-Backward, Viterbi, and Baum-Welch algorithms. This is a somewhat ideal tool for the creation of HMM's that can be manipulated and reused to generate data from ambiguous observation sets. It can be easily installed on any UNIX system and generates output that can be easily stored as desired. Of the three algorithms mentioned, only the Baum-Welch implementation was utilized for this project, as other algorithms relate more to the internal workings of the HMM's and are therefore not of concern to the pure generation of artistic information.

There are two main executables of note from the package that were utilized in this project. The first is called 'esthmm,' which implements the Baum-Welch algorithm and is the HMM generator. It takes for input a number of states, a number of symbols, and an observation filename, which all correspond to the necessities for generating an HMM. It also takes for input an optional random number seed. This guarantees generation repeatability. Utilizing the Baum-Welch algorithm, an HMM is created that is an attempt by the algorithm to find the overall pattern of the data fed to it through the determination of weights in a state machine of a predetermined size (a finite series of nodes and fully connected paths) based on Markov probabilistic mathematics. This represents the foundation of the artistic process for the AI: getting data from nature and finding patterns.

The second executable is called 'genseq,' which generates sequences from an HMM and represents the second step in the artistic process. By generating data from the HMM, an attempt is made to replicate the data that was fed to it. Depending on the nature of the data and the specifics of the HMM initialization, various degrees of accuracy are achieved. Generally, for sufficiently large datasets, true accuracy is not what is sought, due to memory constraints; rather, approximation is sought, and therefore allows for the variability in the creative process described in the Artistic Motivations section. This executable also allows a seed value in its input string to facilitate repeatability.

The second primary software tool is jMusic, an open source, java based, and algorithmic composition software package. As the author accurately describes, "jMusic is a project designed to provide composers and software developers with a library of compositional and audio processing tools. It provides a solid framework for computer-assisted composition in Java, and is also used for generative music, instrument building, interactive performance, and music analysis."[4] This is an extremely useful set of tools, more robust than necessary for this project, yet still intuitive to use and having every resource required.

The structure of jMusic is built around a note object that gets added to successively larger collections of notes within other object structures. These note objects represent a section of memory in the computer that corresponds to all of the features of a single note or rest. These sections of memory are then linked together with other similar sections to make

larger objects in memory that correspond to other musical structures, such as bars, voices, and whole compositions. The primary use for jMusic in this project was to manipulate the data compositionally once processing with the HMM tools was complete.

Compositional Structure

The structure of this piece was designed to make evident to the listener the pattern recognizing and the expressive capabilities of the HMM system. The flowchart in Diagram A is a visual example of the structure. It is a four voice piece built around the data generated by the HMM system overlaid with the original observation data. For clarification, a musical voice is defined here as a single, horizontal line of music that bares some intended relationship to the notes that precede or proceed from any given note in that line. This data is a 22 year collection of sunspot data from 1983 to 2004 gathered from the World Sunspot Index.[5] Sunspots are a series of dark blotches that occur on the surface of the sun and are studied because of the magnetic phenomena surrounding them. Sunspot data was chosen because the spots seem to have a cyclic quality across a 22 year period. Note that the piece was rotated so that it begins in 1996 for aesthetic reasons.

Beginning in 1992, measurement techniques became more advanced, and so there is an increased amount of data starting in this year. Voices 2 and 3 utilize an extra two columns of data provided in the years 1992 through 2004 for the purpose of varied rhythms and dynamics. The pitch material for all of the voices of the piece comes from the first column of data, which runs from 1983 through 2004 (See Diagram B below).

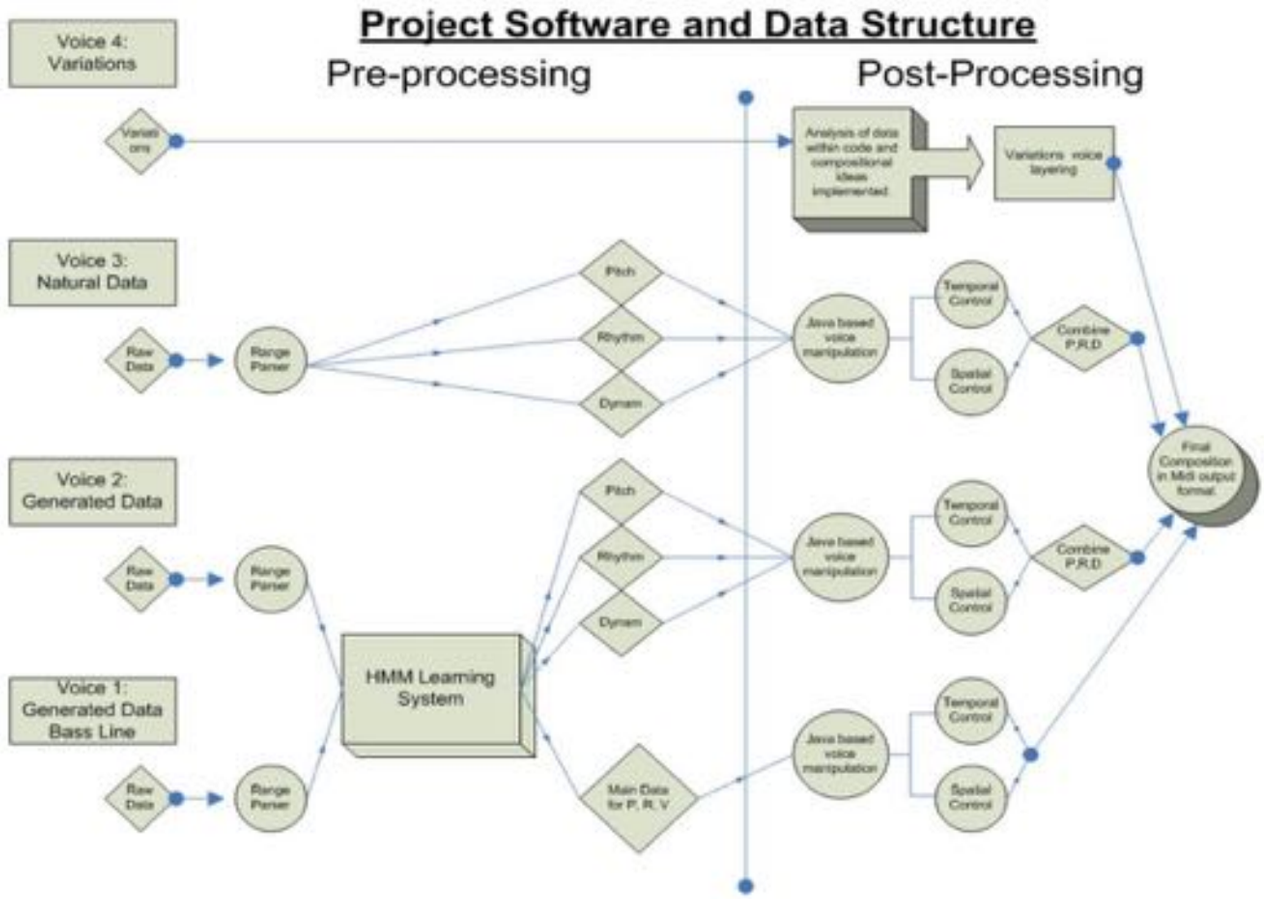
Voice 3 utilizes the original sunspot data as a source for pitch, rhythm, and dynamic material; voice 2 utilizes HMM generated data to determine the pitch, rhythm, and dynamics; the bass line, voice 1 uses HMM data for the pitch material, but the rhythm and dynamics are static. This choice was made so that the listener has some sense of meter, because the piece has specific meter. The variations voice, voice 4, is built over the section of the piece where the material for voices 2 and 3 is incomplete because of the year. While this voice is built off of the same pitch material as the line below it, it utilizes a more staggered structure that is apparent to the listener.

The tempo for the final form of the piece is 5000 beats per minute to facilitate a reasonable overall length across 22 years of data. The scale that was chosen to filter the pitch data through for the final form is pentatonic. It is believed that this scale allows the listener to further appreciate the aesthetic qualities of the piece. The chromatic version, however, facilitates a better sense of the cyclic motion naturally found within the data.

Most of the filtering was done linearly. A change in the data by one notch would affect the pitch, rhythm, or dynamics a

Diagram B: Software Design Flow Chart

The design procedure of the project followed this flow chart. There is an attempt in the creation of this chart to be general and robust enough to facilitate an understanding of the experimental procedure of the project. The line separating the Pre-Processing and Post-Processing sections represent the point at which "compositional" data has been collected from the HMM system and is them brought into the jMusic music processing package. The lone letters P, R, and D correspond to musical Pitch, Rhythm, and Dynamics (note volume) respectively.



single notch where appropriate. All layers of data were kept lined up by day so that, for example, the pitch data in voice 4 for April 3rd, 1995, will temporally line up with the same pitch data in the base line.

In these ways delineated above, the hypothesis is that the listener should be able to hear patterns found by the HMM system both melodically and harmonically.

Program Structure

The program structure is well defined by diagram B shown above. A set of raw data from the sun spots was first parsed into the proper ranges for use in the music. Sunspot data had a range within a given column as high as 246 through 0. This is too great to be applied to the Western pitch system. The 'Range Parser' tool specified in the diagram changes these ranges in a balanced way to protect the cyclic structure of the data, while allowing for a reasonable range to work with compositionally.

Relative to its appropriate voice, the parsed data was then fed through the HMM learning system and saved; then, generation sequences were made based on the HMM's interpretation of the natural data. There was quite a rigorous stage of data manipulation in this section of the project that could have been more easily facilitated by an entirely Java based system.

The proper columns of data were then formatted for use in Java and passed to the proper voice controllers as specified in the previous section. Each voice was generated independently of the others. Temporal and spatial relationships were checked so that the patterns within data would remain intact and the artistic intent maintained. In this stage of the post-processing, the variations voice was compiled and collected with the other voices in preparation for exporting.

jMusic facilitates a convenient conversion of musical data into standard Midi format. Thus a completed piece of music is created. It is important to note for artistic and scientific purposes that all aspects of this project, including all generations and combinations, are repeatable using the methods specified in this paper and the seed value 0605.

Conclusions

Most musically-related work with HMM's is in the areas of transcription and recognition, rather than composition. As this project shows, the potential for an HMM composition system is quite viable and applications for composition are available for expansion. The composer can choose any level of utility for the system, from thorough piece creation, as with this project, to simply using it as a source for compositional material. Future work in this area could be done on building more codependence between the voices and further elaboration on the variations section.

It is clear that an AI system has the potential to be highly expressive, given a sufficient base of experience and an advanced system with which to communicate with human beings.

Acknowledgements

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Molecular Cloning and Characterization of FIERY1 Suppressors

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Light and appropriate responses to it are essential for plant life; as autotrophs, plants need sunlight to generate the food source on which they subsist. The quality and quantity of light also act as environmental signals from which plants derive information to adjust their growth and developmental programs.

Abiotic stresses such as drought and cold stress are another group of environmental signals that may have a dramatic impact on the plant's productivity. Appropriate responses to abiotic stresses are essential; without them, plants would die upon exposure to mild cold or water deficit. It is unclear whether these two kinds of environmental signals interact in regulating plant growth and development. Limited evidence suggests that light, through the action of the phytochrome receptor PhyB, may enhance the induction kinetics of certain cold response genes.[1] However, little else is known about how these two essential signaling systems might overlap in plant signal transduction.

Light plays a key role in such varying stages of plant development as germination, flowering, and direction of growth. In order to use light for these purposes, the plant must have a complicated system of light sensory mechanisms which

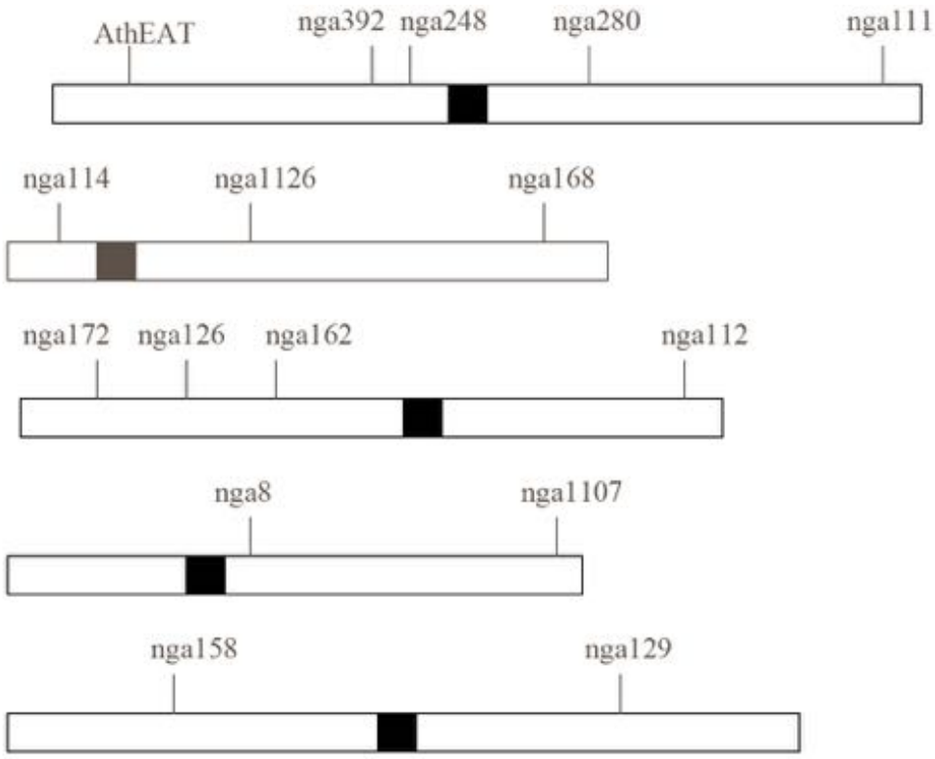

Figure 1: SSLP markers differing between C24 and Columbia ecotypes. Most shown were used in bulk and/or fine mapping.

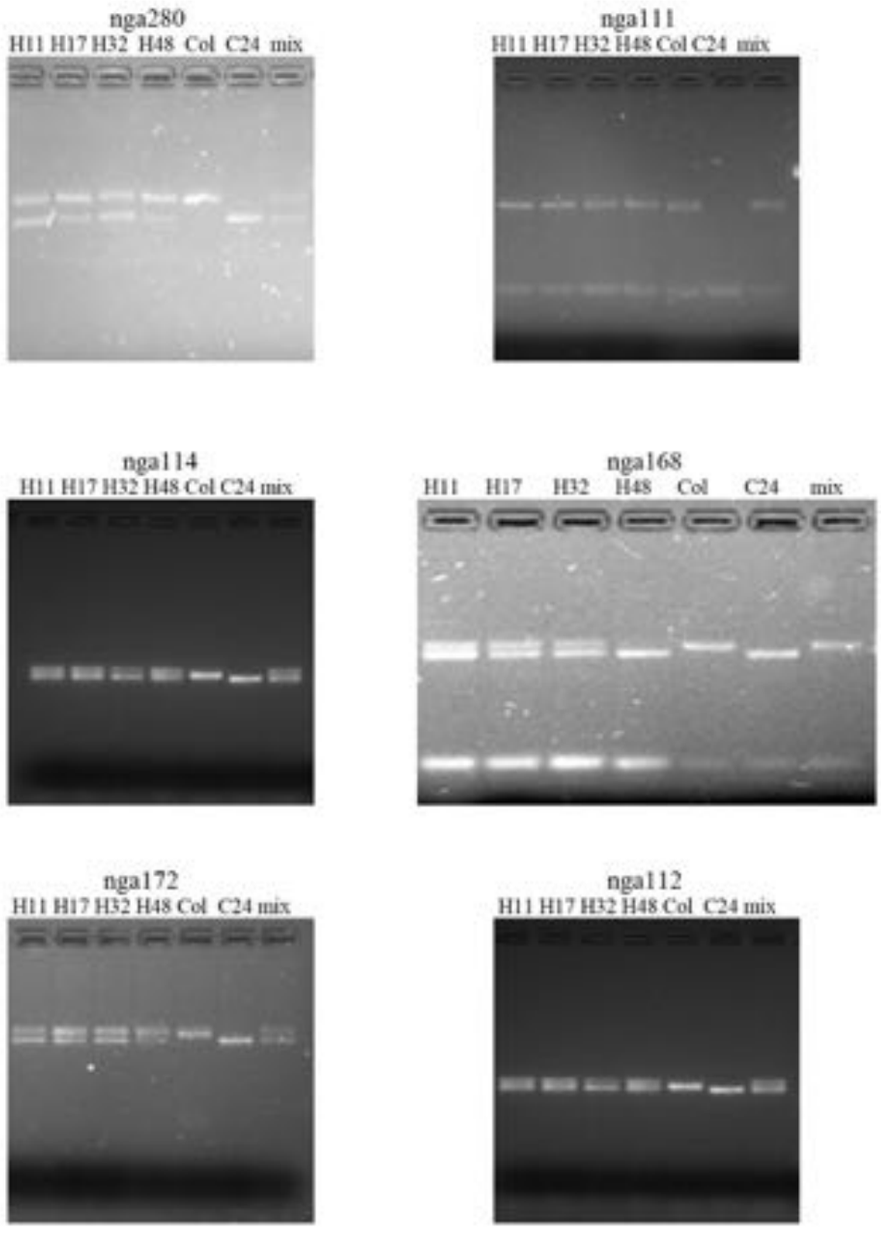

Figure 2: Bulk segregant analysis of chromosomes one, two and three.

help initiate a response. Four major types of photoreceptors have been described in Arabidopsis: UVB photoreceptors, which are still uncharacterized; cryptochromes, which respond in blue and UVA light; phototrophins, which respond in blue light; and phytochromes, which respond in red and far-red light. Arabidopsis employs five phytochromes (known as PhyA-E) that belong to two types: type one is light labile, while type two is light stable, though all are expressed throughout the plant. The type one phytochrome PhyA is downregulated and the protein is degraded upon exposure to light and is therefore present mostly in dark-grown seedlings. On the other hand, PhyB, the most abundant of the type two phytochromes, is very light stable.[2,3]

In its first stages of growth, Arabidopsis is uniquely sensitive to its light environment, for the amount of light determines how the hypocotyl and cotyledons will develop, and much of this sensitivity is determined by phytochromes. Normally, in a light-free environment such as the soil, hypocotyls grow long and cotyledons remain closed due to the Very Low Fluence Response (VLFR) initiated by PhyA. When full light is present, the hypocotyl is short and cotyledons open wide primarily due to PhyB action, although PhyA and PhyD have also been implicated in the inhibition of hypocotyl elongation.[2,3]

PhyB also operates later in the plant's growth cycle through the shade avoidance response. PhyB is very sensitive to the differences between red and far-red light, and hence serves as a monitor of the red/far-red ratio in Arabidopsis. Green plant material reflects light rich in green and far-red, but lacking in

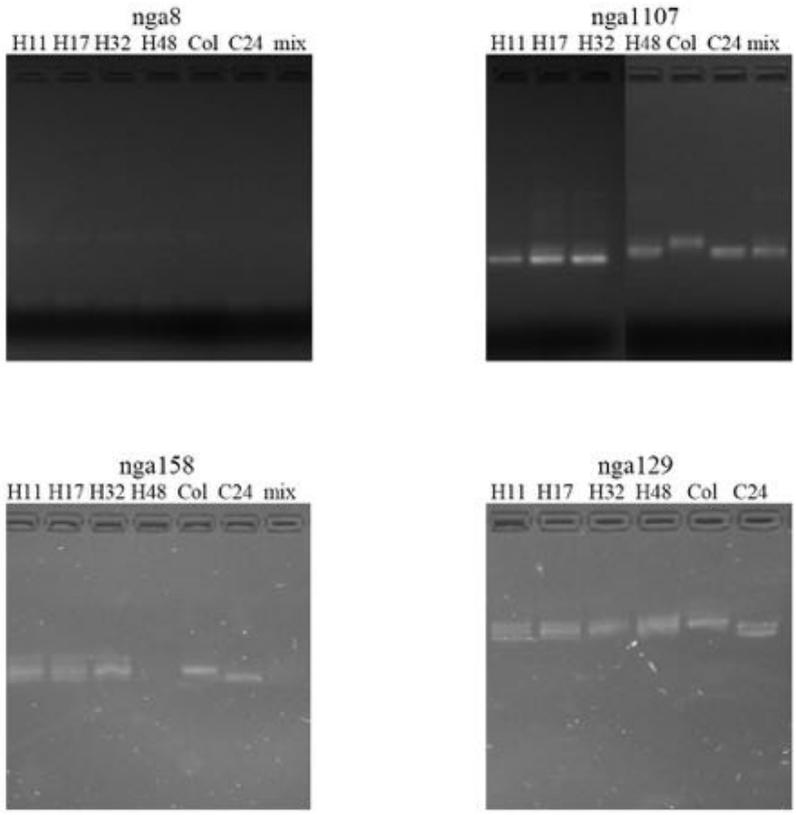

Figure 3: Bulk segregant analysis of chromosomes four and five.

blue and red; therefore a low red/far-red ratio as detected by PhyB indicates shading by other plants. In order to avoid being stifled by neighboring plants, Arabidopsis sensing this low ratio initiate the shade-avoidance response, usually involving elongation of the stem and petioles and early flowering.[4]

To isolate genes important for stress signal transduction, the Xiong lab used a firefly luciferase reporter gene system to conduct genetic screens. The reporter gene is comprised of the well-known bioluminescent luciferase gene fused to an RD29a promoter, which contains both the dehydration response element (DRE) that responds to cold and osmotic (drought) stress and the abscisic acid (ABA) responsive element (ABRE) that responds to the phytohormone abscisic acid.5 When introduced into Arabidopsis, this gene functions as a reporter of stress-responsive gene induction. The degree of luminescence measures the strength of the stress response. Using this system, a genetic locus, FIERY1, was identified whose mutations resulted in much higher luminescence than in the wild type upon exposure to cold, osmotic stress and ABA, indicating hyperinduction of stress-responsive genes. The fiery1 mutants also exhibited decreased tolerance to cold, drought, and salt. Surprisingly, fiery1 mutants were also altered in their photomorphogenic responses, having short hypocotyl length and wide cotyledon angle when placed in dim light.

The FIERY1 (abbreviated FRY1) gene encodes a phosphatase that functions both as a 3'(2'), 5'-bisphosphate nucleotidase and as an inositol polyphosphate 1-phosphatase. FIERY1 activity is responsible for inositol 1,4,5-tirsphospate (IP_3) catabolism in Arabidopsis. IP_3 is thought to function in the movement of Ca^{2+} ions, a well-known participant in many signal transduction cascades, by initiating its release from vacuolar stores.[6]

Based on the photomorphogenic effects of the fiery1 mutation, we hypothesize that calcium ions, and therefore IP_3 and FRY1, play important roles in plant light response. The Xiong lab has identified 54 fiery1 suppressor mutant lines comprising 12 loci. Four of these loci recovered wild type hypocotyl length while maintaining cold, ABA, and drought hypersensitivity. This study focuses on cloning the suppressor mutation in four mutants displaying such phenotypes. Interestingly, at least three of these four mutants also display an early flowering phenotype similar to that seen in constitutive shade avoidance. By using simple sequence length polymorphism (SSLP) marker-based mapping strategies (Table 1, Figure 1), we mapped and identified this suppressor gene. The current study should provide an entry point to gain further insight into the exact roles of FRY1, IP_3, and Ca^{2+} in stress signaling and photomorphogenesis.[7]

Materials and Methods

Plant materials

Arabidopsis containing the fiery1-1 (fry1-1) mutation in the C24 ecotype background were mutagenized with ethyl methane sulfonate (EMS). fiery1 suppressors were identified by their longer hypocotyls under dim light. Four homozygous suppressor lines, H11, H17, H32 and H48, were used here for further analysis. These lines were crossed with a fiery1 mutant in the Columbia (Col) background, and the F_1 plants selfed and

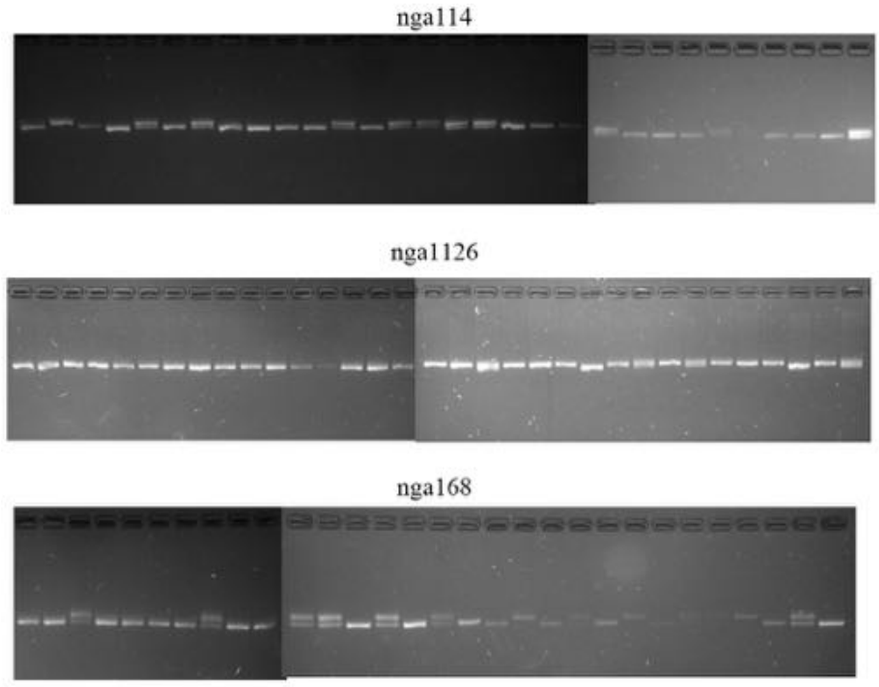

Figure 4: Fine mapping of H11 using chromosome two markers

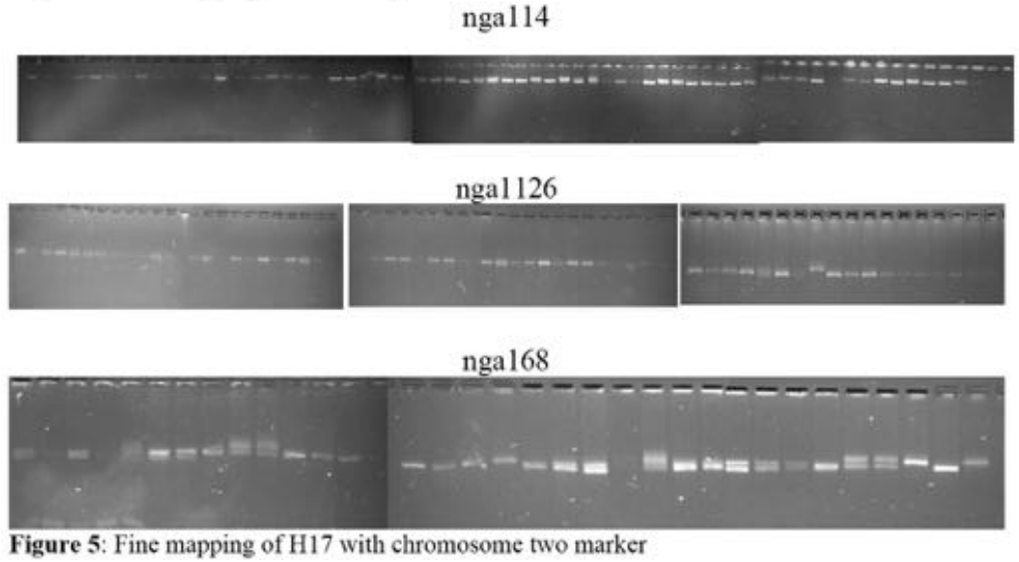

Figure 5: Fine mapping of H17 with chromosome two marker

F_2 seeds collected. These F_2 seeds were planted in a tray with a small section of the original mutant line and dark germinated for 3 days in cold before being placed in the growth chamber.

When the seedlings were 10-days old, homozygous suppressor mutants were identified by their long hypocotyls, a trait easily seen among the F_2 populations. These mutants were harvested for DNA extraction.

DNA extraction and PCR protocol

Similar to the protocol of Edwards et al,[8] each sample of plant tissue was ground with a disposable pestle for 15 seconds and mixed by vortexing with 700 ul of extraction buffer containing 200 mM Tris-Cl (pH 7.5), 250 mM NaCl, 5% EDTA (pH 8.0), and 0.5% SDS. The tubes were then centrifuged at 13000 rpm for 5 minutes, and the supernatant was collected in a new tube and was mixed with 700 ul isopropanol for DNA precipitation at -20°C for at least two hours. The sample was then was centrifuged for seven minutes and the pellet washed with 75% ethanol. After drying in a Vacufuge for five minutes, the pellet was resuspended in 100 ul sterile water.

Extracted DNA was amplified using the following PCR program: 94°C for three minutes, followed by forty cycles of 94°C for thirty seconds, 55°C for thirty seconds, and 72°C for forty five seconds, then ten minutes at 72°C and holding at 4°C. Amplification of the PhyB SSLP marker was accomplished using the same protocol, except that annealing occurred at 57°C. The products were then run on 4% agarose gels at a current between 150 and 250 volts.

Positional Cloning (from Jander et al, 2002)[9]

Bulk samples were assembled by combining 3 ul of DNA from each extracted mutant sample. These samples were run in PCR with several SSLP markers, usually two to three from each chromosome. Band lengths and intensities were compared with

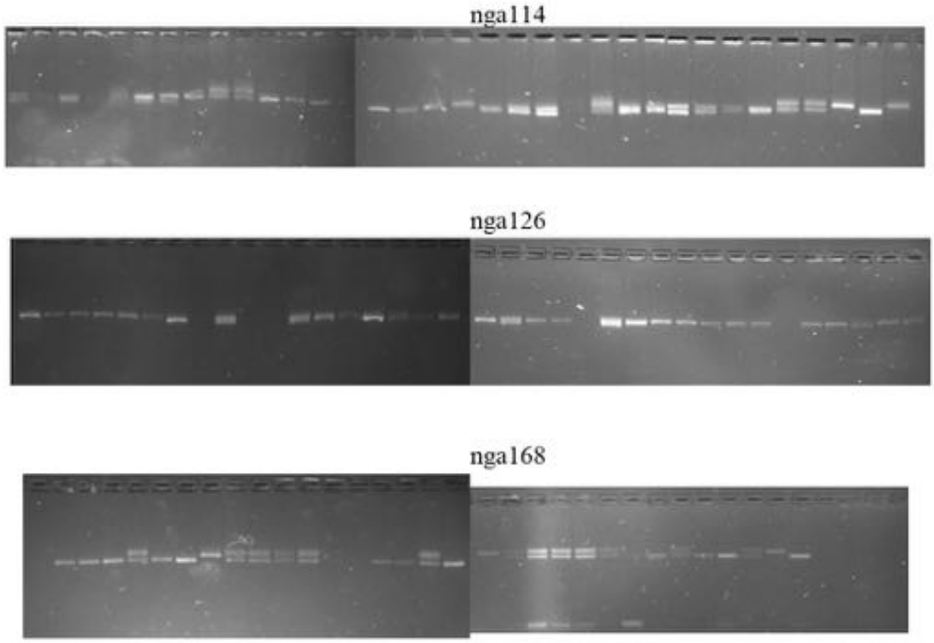

Figure 6: H32 fine mapping with chromosome two markers.

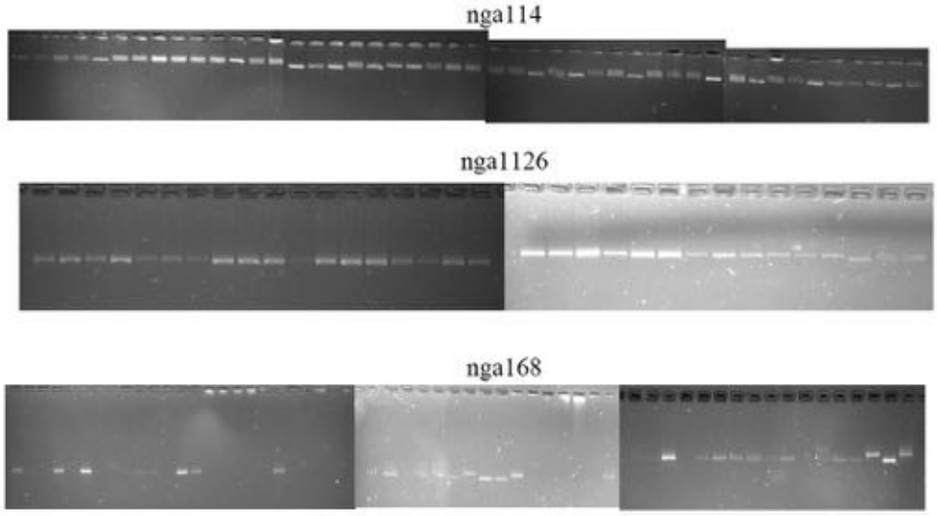

Figure 7: H48 fine mapping with chromosome two markers

the C24 and Columbia ecotype controls to determine linkage in each mutant. If bulk segregant analysis was not successful in determining chromosome linkage, individual samples from each mutant would be run with the same SSLP markers, and percent recombination, calculated as

$$\frac{2(\#\text{ double recombinant}) + 1(\#\text{ single recombinant})}{2(\text{total }\#\text{ samples})},$$

was used to estimate the mapping distance.

Once the mutant was mapped to a particular chromosome, fine mapping was carried out. This was done by amplifying individual DNA samples with appropriate SSLP markers from that chromosome and narrowing the region by "chromosome walking." Genes from this delimited interval were then amplified and sequenced. The sequence from the mutant was compared to genomic DNA of the wild type to locate the mutation site.

PhyB and Hy1 Gene Sequencing

In the case of H17, H32 and H48, DNA samples that were isolated from soil-grown seedlings were amplified with PhyB primers using the PCR program: 3 minutes at 94°C followed by 36 cycles of 30 seconds at 94°C, 35 seconds at 56°C, and 1 minute at 72°C, ending with 10 minutes at 72°C and holding at 4°C.

For H02, H03, H44, H45, and H46, seeds were sterilized and planted on 0.6% Murashige and Skoog (MS) basal media, allowed to sit in the cold room for 3 days, and then placed in the growth chamber for 6 days. Seedlings were then harvested and DNA extracted using the above protocol, and the DNA was amplified with PhyB primers according to the above program.

H11 DNA extracted from soil-grown seedlings was amplified with Hy1 primers using the following PCR program: 3 minutes at 94°C followed by 36 cycles of 45 seconds at 94°C, 50 seconds at 54°C, and 1 minute 3 seconds at 72°C, ending with 10 minutes at 72°C and holding at 4°C.

All PCR products were purified using the Qiagen kit and sent to Lark Technologies for sequencing.

Results and Discussion

Bulked segregant analysis of H32, H17, H48, and H11 (Fig 2 and 3; Table 2) indicated that all four mutations localized to chromosome two. Fine mapping with three markers from chromosome two (nga1145, nga1126 and nga168) was initiated (Figures 4-7). Nga1126 showed a recombination frequency of 11.67% in H11, 12.22% in H17, 8.70% in H32, and 2.86% in H48 (Tables 3-6). Such low recombination frequencies indicated tight linkage to this marker, which is in close proximity to two possible genes of interest: PhyB and Hy1.

Previous studies have indicated that PhyB plays a significant role in flowering time, with PhyB-deficient mutants flowering well before the wild type. It has been suggested that PhyB posttraslationally regulates Constans (CO), a transcription factor that controls Flowering Locus T (FT), which normally acts to induce flowering. And indeed, FT transcript is more abundant in phyb mutants, which easily explains the early flowering phenotype.[4,10] Since three of the four suppressor mutations flowered earlier than the fiery1 mutant (which has a late flowering phenotype), it seems likely that an inactive or truncated PhyB protein might be responsible for their mutant phenotype.

To determine whether there is any mutation in the PhyB gene, the PhyB gene from H32 was amplified and sequenced. After initial results indicated a possible mutation within the gene, C24 wild type DNA was also sent for sequencing so that the mutation could be confirmed or ruled out as a polymorphism between ecotypes. The C to T substitution within the first

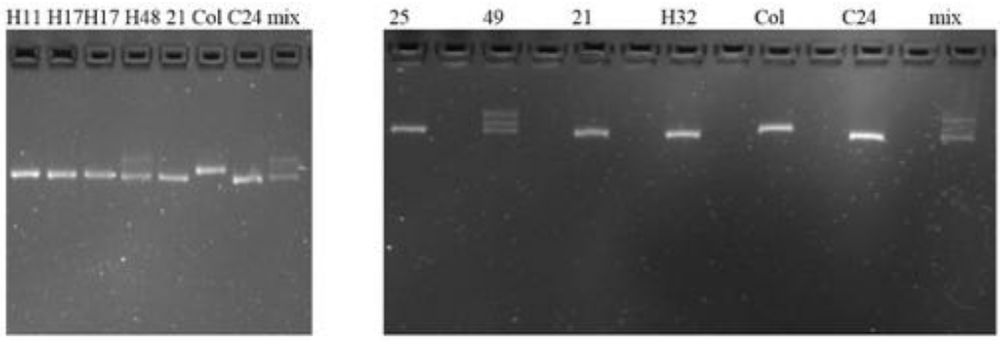

Figure 8: Bulk analysis using newly designed PhyB SSLP primer. 21, 25, and 49 are sample numbers for negative controls, samples which showed recombination at all three chromosome two markers. Only 49, an H17 sample, proved to be useful in this role.

Figure 9: Sequencing data comparing the H32 PhyB mutant sequence to wild type Columbia CDNA. The T outlined in white is the mutation site, which turns an GAG codon to a UAG stop codon.

Figure 10: Sequencing data comparing H17 PhyB mutant (bottom row) with wild type Columbia cDNA and genomic DNA from both Columbia and C24. The A outlined in white is the mutation site, which turns a AGU codon for serine to an AAU codon for asparagine.

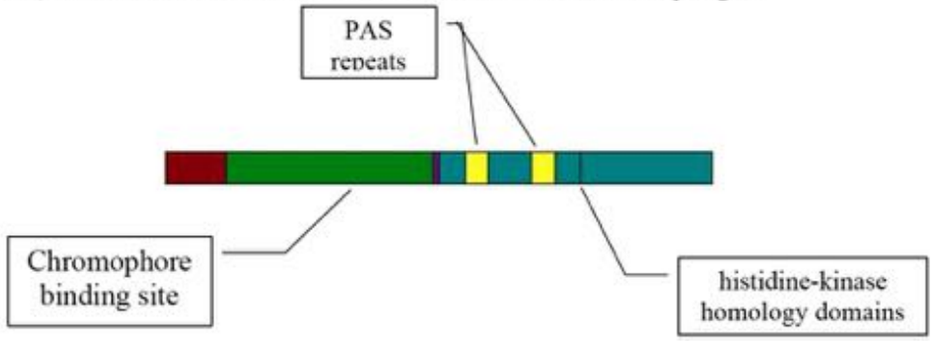

Figure 11: Domain structure of phytochromes. The purple segment in the middle corresponds to a "hinge" between the N-terminal half and the C-terminal half, which vary greatly in structure.

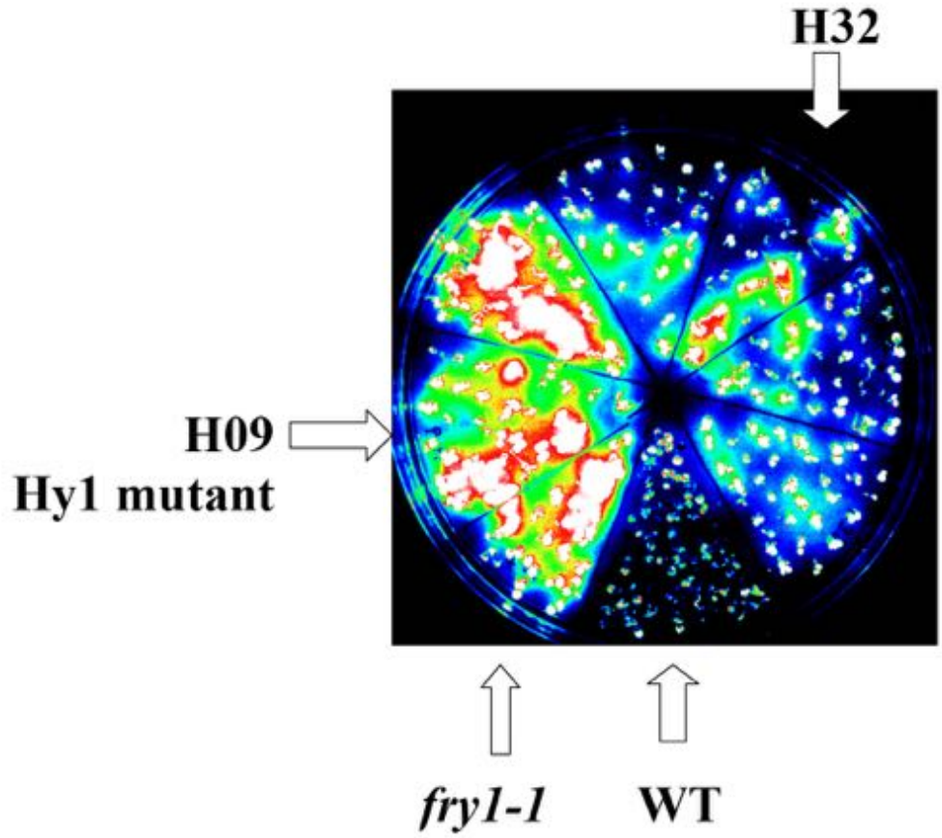

Figure 12: Bioluminescence of the firefly luciferase gene attached to the RD29A promoter in H32, a Hy1 mutant, fry1-1 and wild type seedlings.

	Forward Sequence	Reverse Sequence
nga248	5' - TCTGTATCTCGGTGAATTCTCC - 3'	5' - TACCGAACCAAAACACAAAGG - 3'
nga280	5' - GGCTCCATAAAAAGTGCACC - 3'	5' - CTGATCTCACGGACAATAGTGC - 3'
nga111	5' - TGTTTTTTAGGACAAATGGCG - 3'	5' - CTCCAGTTGGAAGCTAAAGGG - 3'
nga114	5' - CCTTCACATCCAAAACCCAC - 3'	5' - GCACATACCCACAACCAGAA - 3'
nga1126	5' - GCACAGTCCAAGTCACAACC - 3'	5' - CGCTACGCTTTTCGGTAAAG - 3'
nga168	5' - GAGGACATGTATAGGAGCCTCG - 3'	5' - TCGTCTACTGCACTGCCG - 3'
nga172	5' - CATCCGAATGCCATTGTTC - 3'	5' - AGCTGCTTCCTTATAGCGTCC - 3'
nga112	5' - CTCTCCACCTCCTCCAGTACC - 3'	5' - TAATCACGTGTATGCAGCTGC - 3'
nga8	5' - TGGCTTTCGTTTATAAACATCC - 3'	5' - GAGGGCAAATCTTTATTTCGG - 3'
nga1107	5' - CGACGAATCGACAGAATTAGG - 3'	5' - GCGAAAAAACAAAAAAATCCA - 3'
nga158	5' - ACCTGAACCATCCTCCGTC - 3'	5' - TCATTTTGGCCGACTTAGC - 3'
nga129	5' - CACACTGAAGATGGTCTTGAGG - 3'	5' - TCAGGAGGAACTAAAGTGAGGG - 3'

Table 1: SSLP primers used in bulk and fine mapping.

	marker	H11	H17	H32	H48	
Chrom 1	nga248	?	?	?	?	X = C24
	nga280	*	*	*	*	O = Col
	nga111	?	?	?	?	* = recom
chrom 2	nga114	X	X	X	*	
	nga168	X	*	*	X	
chrom 3	nga172	*	*	*	*	
	nga112	*	*	?	*	
chrom 4	nga8	?	?	?	?	
	nga1107	?	?	?	?	
chrom 5	nga158	*	*	*	?	
	nga129	*	*	*	*	

Table 2: Bulked segregant recombination scoring

H11			
	nga1145	nga1126	nga168
1	X	X	X
2	O	*	X
3	X	X	*
4	X	X	X
5	*	X	X
6	X	X	X
7	*	X	X
8	X	*	*
9	X	X	X
10	X	X	X
11	X	X	*
12	*	X	*
13	X	X	X
14	*	X	*
15	*	X	X
16	*	X	*
17	*	X	X
18	X	X	X
19	X	*	O
20	X	X	X
21	*	X	*
22	X	X	X
23	X	O	*
24	X	X	X
25	*	*	*
26	?	X	*
27	X	*	O
28	X	X	X
29	X	X	*
30	*	X	X
% recom	20.68966	11.66667	25

Table 3: H11 recombination mapping data

1000 base pairs of the gene initially noted in H32 was, in fact, not present in either Columbia or C24 wild type DNA. This mutation would lead to the change of a glycine-coding CAG codon to the stop codon AUG and would result in a truncated PhyB protein that is most likely nonfunctional. Therefore, H32 is most likely a null allele of PhyB (Figure 9).

Interestingly, while sequencing the H32 and C24 PhyB gene, we discovered an unreported polymorphism between C24 and Col in the PhyB gene. The 12 base pair sequence after nucleotide 41 (in cDNA) in Columbia is absent in the C24 ecotype. Using this polymorphism, we generated a new SSLP marker within that gene. Using the already-made PhyB 1F sequencing primer and a reverse primer designed after the discovery of this marker, the four suppressor mutants showing linkage to chromosome two were tested for their linkage to PhyB. Bulk samples of all the unsequenced mutants, originally used in bulk segregant analysis, were amplified with the new primers (Figure 8). A negative control, a single DNA sample which had shown recombination at nga114, nga1126 and nga168 in fine mapping, was used, and it confirmed that the marker is reliable. Both H11 and H17 showed only a single band, corresponding to the C24 segment. H48, however, showed two bands. Although the C24 band is obviously stronger, this still calls into question the identity of the H48 mutation.

Since H17 and H48 had been mapped to the region on chromosome two containing PhyB (Tables 5 and 7) and displayed similar phenotypes (e.g. early flowering) to the mutant H32, both mutants were sequenced for PhyB.

Indeed, a mutation in PhyB was detected in the H17 mutant. This G to A substitution near position 2600 of the genomic DNA would convert an AGU codon for serine to an AAU codon for asparagine (Figure 10). This alteration occurs in the carboxyl half of the protein, a region composed of two bacterial histidine-kinase domains. The first of these domains contains two PAS repeats, and the H17 mutation occurs within the second of these repeats (Figure 11). Although serine and asparagine are both small, polar, and structurally similar, serine phosphorylation has been suggested to be essential for Phytochrome A function and may also be important in that of Phytochrome B. Asparagine cannot be phosphorylated, and it is this difference that may be the actual cause of the aberrant phenotype.

H11, having initially been sequenced for Hy1 only to show no mutation, has not yet been sequenced for PhyB, but the SSLP results suggest that this gene could be the site of that mutation.

Such conclusive linkage of these fiery1 suppressors to phytochromes, which are essential to light signaling, indicates that FRY1 represents a point of crossover between ABA-independent (such as cold) and ABA-dependent stress signaling and the light signaling of photomorphogenesis. Indeed, some evidence already exists for the overlap of cold signaling and light signaling through Phytochrome B. Kim et al (2002)[1] used GUS staining and null mutants to show that light, specifically Phytochrome B activity, is essential for induction of cold and drought responsive genes linked to the ABA-independent C/DRE promoter. Also, Halliday et al (2003)[4] showed that the early flowering phenotype of phyB mutants was abolished at temperatures below 16°C, though fully apparent at 22°C. Although they also implicated PhyE in this process, their results still indicate yet another connection between photomorphogenesis and stress response.

One possible component underlying this cross talk could be Ca^{2+}. Ca^{2+} has already been implicated as an important second messenger in cold stress response. Interestingly, UVA/Blue Light and UV-B have been shown to regulate calcium levels in the cytosol.[11] FRY1 itself may regulate calcium ion flow through IP_3 catabolism and, through this, mediate the stress response.[6] Therefore it seems likely that fiery1 suppressors, and therefore PhyB, should be related somehow to the ubiquitous calcium signal.

The cross-talk could also be mediated by Phytochrome B itself through its particular domain. The two PAS repeats in Phytochrome B are known to play roles in signaling through protein-protein interactions and responses to small ligands or changes in light, redox potential, or oxygen levels.[12] These PAS repeats are hotspots for missense mutations which alter, but generally do not abolish, the function of PhyB, causing speculation that they are used in interactions with downstream signaling molecules.[13] H17, whose mutation occurred within the second of these PAS repeats, has a phenotype unique among the studied fiery1 suppressors in that it displays a heightened sensitivity to ABA, even above the initial hypersensitivity of fiery1. This suggests that the PAS repeats in PhyB may play a

H17			
	nga1145	nga1126	nga168
1	X	X	X
2	X	-	*
3	X	X	-
4	X	X	-
5	X	*	-
6	X	*	-
7	-	X	-
8	*	-	-
9	X	-	O
10	X	X	*
11	-	*	*
12	X	-	-
13	*	X	X
14	X	*	*
15	X	-	*
16	*	X	X
17	*	X	*
18	*	X	*
19	X	X	X
20	X	X	-
21	X	*	-
22	-	X	-
23	-	-	-
24	*	X	-
25	*	-	*
26	*	X	X
27	*	X	X
28	X	X	-
29	*	X	-
30	*	X	X
31	*	X	X
32	X	X	-
33	X	X	X
34	*	X	-
35	X	*	-
36	*	X	X
37	*	X	-
38	X	X	*
39	X	*	*
40	X	X	*
41	X	X	X
42	*	X	X
43	*	X	X
44	X	X	X
45	X	X	X
46	X	X	-
47	X	*	O
48	*	O	X
49	*	*	*
50	O	X	*
% recom	22.34	12.222	24.194

Table 4: H17 Recombination mapping data

role in ABA signal transduction.

However, purely genetic studies such as this one cannot conclusively show the interactions of signaling proteins or the role of an inorganic ion such as calcium in these signaling pathways. Further studies should be carried out to determine whether or not there are ABA-signaling proteins that interact with PhyB and the nature of that interaction. ABA-dependent and -independent pathways could also be studied to determine if there are differences in their relations to phytochromes, since this study only shows a general overlap between stress signaling and light signaling.

Also, there are many other fiery1 suppressor mutants that have not been studied or sequenced; even H11, though it showed linkage to PhyB, has not been fully analyzed. Further study of these mutations could yield even more information concerning the roles of phytochromes and other light signaling molecules.

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H32			
	nga1145	nga1126	nga168
1	X	X	?
2	X	X	X
3	X	X	X
4	?	X	X
5	?	X	*
6	?	X	X
7	X	?	X
8	?	*	O
9	X	*	*
10	?	X	*
11	?	?	*
12	?	?	*
13	X	?	?
14	X	?	?
15	X	X	X
16	O	X	X
17	X	*	*
18	*	X	X
19	?	X	O
20	X	?	*
21	*	*	*
22	X	X	*
23	X	X	*
24	X	X	*
25	*	X	X
26	X	X	X
27	X	X	X
28	*	?	X
29	O	X	X
30	X	X	*
% recom	18.18182	8.695652	29.62963

Table 5: H32 recombination mapping data

H48			
	nga1145	nga1126	nga168
1	X	X	X
2	*	X	X
3	*	X	X
4	*	X	X
5	X	X	*
6	*	X	X
7	*	X	X
8	*	X	O
9	*	X	O
10	*	X	X
11	*	X	X
12	O	X	X
13	*	X	X
14	*	X	*
15	-	X	-
16	X	X	X
17	X	X	X
18	X	X	X
19	*	X	*
20	X	X	X
21	X	X	X
22	X	X	X
23	*	X	X
24	*	X	X
25	*	X	X
26	*	X	X
27	*	X	X
28	X	X	O
29	*	X	X
30	X	X	X
31	*		X
32	*		*
33	X		X
34	*		X
35	*		X
36	*		X
37	X		X
38	*		X
39	X		X
40	*	X	X
41	*	X	X
42	X	X	*
43	X	*	X
44	X	*	X
% recom	33.33333	2.857143	12.7907

Table 6: H48 recombination mapping data

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Learning and Children's Theory of Gravity

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Introduction

By 24 months of age children can comprehend that during occlusion, objects exist and can be moved to hidden locations.[3] The development of such spatial reasoning in young children has been explored by a number of tasks that involve novel invisible displacements. These tasks obscure an object (e.g., a ball) in a container, and the object is subsequently moved to a number of possible hiding places.

More recent research has shown that infants as young as 4 months of age have some understanding of object permanence, gravity displacement, solidity of objects, and continuity of objects in motion. This has been established by demonstrating that the infants expect an unsupported object to fall in a straight trajectory unless obstructed by a solid obstacle.4 However, it has also been shown that 2- and 3-year-old children cannot use knowledge about the way obstacles constrain motion to predict an object's trajectory.[1]

Hood examined the spatial skills of 2 to 4 and 1/2 year olds by constructing complex invisible displacement tasks where a ball was dropped into an opaque tube that led to one of three possible hiding places.[1] However, the tubes were never arranged in a vertical orientation; therefore, the ball was never found directly below the tube's entryway. The tubes redirected the path of the ball so that the ball's final location was not the result of a straight-down trajectory due to gravity. Hood found that task performance was significantly related to tube complexity; older children solved arrangements with more tubes than younger children. He also found that the children's search errors were consistently aimed at the location directly below the location where the ball was inserted into the tube, and this pattern of response was called the "gravity error."

Subsequent studies by Hood tested children's naïve concept of gravity and confirmed that young children are inclined to believe that falling objects travel in a straight line.[2] However, children did not show an anti-gravity error when shown a videotape that played the event backwards (the ball appeared to move up the tube rather than down the tube). Taken together, these findings suggest that young children believe the naïve theory of gravity regarding falling objects, and this theory overrides the cues provided by the tubes that would violate the gravity bias. With age and experience, children seem to experience a progressive awareness of the way tubes restrict the movement of a falling object.[1,2]

The goal of the present study was twofold. The first was to investigate whether children who were allowed to watch an adult make both correct and incorrect responses on an invisible displacement task would perform better compared to children who did not receive this observational training. The children in the non-observational group simply continued to perform the search task without assistance. We predicted that performance would increase in both groups of children but that the observational group would show a greater increase in performance during the test phase. The second purpose of the study was to determine whether or not the majority of children's first-search errors were in fact gravity errors. We predicted that the majority of first-search errors would be aimed towards locations directly below the ball's entryway into the tube and could therefore be classified as gravity errors.

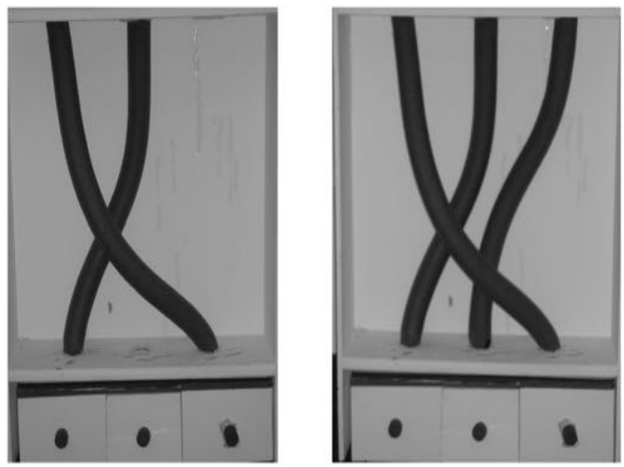


Figure 1: Apparatus configurations showing difficulty level 2 (left) and level 3 (right).

To investigate these hypotheses, we tested children between 3 and 5 years of age using Levels II and III of Hood's tube configurations (see Figure 1) and recorded the children's responses, including their specific search patterns.[1] Hood's

findings show that children 24 months of age and older are able to pass level II of the opaque tube experiment, in which there are only two alternative tube locations, but are unable to pass level III, in which there are three tube locations.[1] All children in the present experiment first had to pass level II to ensure they had the capacity to correctly retrieve the object. All subsequent testing involved level III, which has been shown to be very difficult for 2- to 4-year olds. After an initial set of test trials at level III, we randomly assigned the children to either the watch-learning group or the self-learning (control) group. Children in the watch group observed an experimenter correctly find the ball after being dropped down each of the three tubes, while children in the self group simply continued to perform an additional set of trials at level III without experimenter interference. After the learning trials, the configuration of the three tubes was changed to ensure that the children were not simply memorizing the path of the tubes. The children were then asked to perform a post-learning test phase with this new tube configuration. The measure of interest was the improvements in correct first search locations in the post-learning test trials compared to the pre-learning test trials.

Method:

Participants

Thirteen children ranging in age from 36 to 59 months participated in the experiment. However, the results of four children were not included in the study; three children failed to pass Level II, and a fourth child scored perfectly on the Level III pre-test and therefore could not demonstrate a learning curve. Of the remaining nine children, there were five males with a mean age of 47.8 months (SD=9.07) and four females with a mean age of 52.9 months (SD=4.77). The participants represented a range of races and socioeconomic classes. The children were recruited at a local daycare center through written consent forms, which were distributed to parents whose children attended the daycare. There was no payment in exchange for participation in the experiment; however, children were given stickers as a reward for their participation regardless of the accuracy of their responses. The children were treated in accordance with the "Ethical Principles of Psychologists and Code of Conduct."[5]

Apparatus

The apparatus, which was made out of wood and painted white, was very similar to the one used by Hood.1 The width of the apparatus was 53.5 cm and the height was 69 cm. The height of the distance that the tubes actually traveled was 50.5 cm. The top of the frame had three holes; each hole led to the hiding box directly below. Each hiding box was 16 x 11 x 11 cm and had a circular hole in the top (Figure 1). The hiding boxes were also painted white and were insulated with padding to prevent the sound of the ball from influencing the children's responses. Each entryway was connected to a nonaligned hiding box by the length of one of the tubes. The three tubes were constructed from grey, flexible, foam tubing and were connected to a given hole by Velcro and yellow duct tape. The diameter of the tubes was approximately 2.5 cm, and the horizontal separation between the tubes was 10.5 cm. The foam material of the tubes also prevented any sound of the ball's movement from influencing the children's responses. The plastic, colored balls (blue, red, pink, green, and orange) were 1.8 cm in diameter. The color of the ball used during the experiment was chosen according to each child's individual preference.

Design and Procedure

Each child was tested in an isolated area of his or her assigned classroom with two experimenters present. One of the experimenters performed the experiment while the other recorded the children's responses. The apparatus, child, and experimenters were seated on the floor. Prior to testing, children were allowed to play with a sample piece of tubing and one of the balls in order to become familiar with the materials and to demonstrate that the tubes were hollow. The child was never shown a vertical arrangement of the tubes. Throughout testing, experimenters were careful to use consistent, objective language with the participants to avoid any biasing of the data.

The first portion of the formal experiment tested the children on Level II of Hood's tube configurations.[1] On this trial and all subsequent trials, excluding training, either the child or the experimenter dropped the ball into one of the tubes. Once the ball had been dropped, it was out of sight until it was retrieved. After the ball had been dropped, the children searched until they found the ball in one of the hiding boxes. The pattern of each child's search behavior was recorded by an experimenter. Each child completed five trials on Level II. If they found the ball correctly (on their first try) on four or more of the five trials, they proceeded to the next level. If a child failed to find the ball correctly on four or more trials, they did not proceed to level III, and their responses were not included in the results. This is based on the established notion that children who are unable to pass Level II (the "easier" level) would subsequently be unable to pass the more complex Level III.[1]

The second portion of the experiment involved Level III of Hood's tube configurations (see Figure 1).[1] There were three separate phases at Level III; a "pre-test", "training", and "test" phase. Each phase included five trials, resulting in a total of 15 trials.

Each child's search pattern was recorded by an experimenter. For the training phase, children were randomly assigned to either the "watch" or "self" group. Four children were included in the "watch" group, and five children were included in the "self" group. Children in the "watch" group completed the training phase by watching the experimenter both drop and find the ball. The accuracy with which the experimenter found the ball varied; on some occasions the ball was found on the first attempt, while on other occasions it was not. The purpose of this was to demonstrate both correct and incorrect responses to the child. If incorrect responses had not been shown, the child might have assumed that a ball could be found in any location, and the training phase would therefore be ineffective. On the other hand, children in the "self" group were instructed to both drop and find the ball themselves. Therefore, this group of children did not receive direct training from the experimenter.

For the test phase, the experimenter reconfigured the tubes to produce a mirror image of the previous configuration. This was done to make sure that the child had to reassess the change of the tubes' arrangement. The children's search patterns were recorded by a second experimenter for each of the trials.

Scoring

The observing experimenter scored each participant's responses. For each trial, the location (left, middle, or right) of the opening through which the ball was dropped and the search locations (correct, incorrect gravity, or incorrect irrelevant) were recorded. The scoring process involved comparing the individual participant's pretest trial score and test trial score. A score was given as the number of correct searches on the first attempt out of each set of five trials. Each child was then given an overall learning score that was obtained by subtracting the pre-test score from the test score. Each individual's score from the "watch" and "self" groups were then averaged together for subsequent analyses.

Results

Overall, there was no statistically significant change from the pre-test score to the test score within participants for either the "self" group ($t(4)=0.31$, $p=0.772$), the "watch" group ($t(3)=1.67$, $p=0.194$), or the groups combined ($t(8)=1.02$, $p=0.336$). A small drop in mean score was seen in both groups, with the "self" group score dropping by 1.25 (SD=1.5) and the "watch" group dropping by 0.4 points (SD=2.88). An unpaired t-test comparing these score changes shows no significant result ($t(7)=0.53$, $p=0.612$) (see Figure 2). There is also no significant correlation between age and performance with or without inclusion of the four participants eliminated from the study.

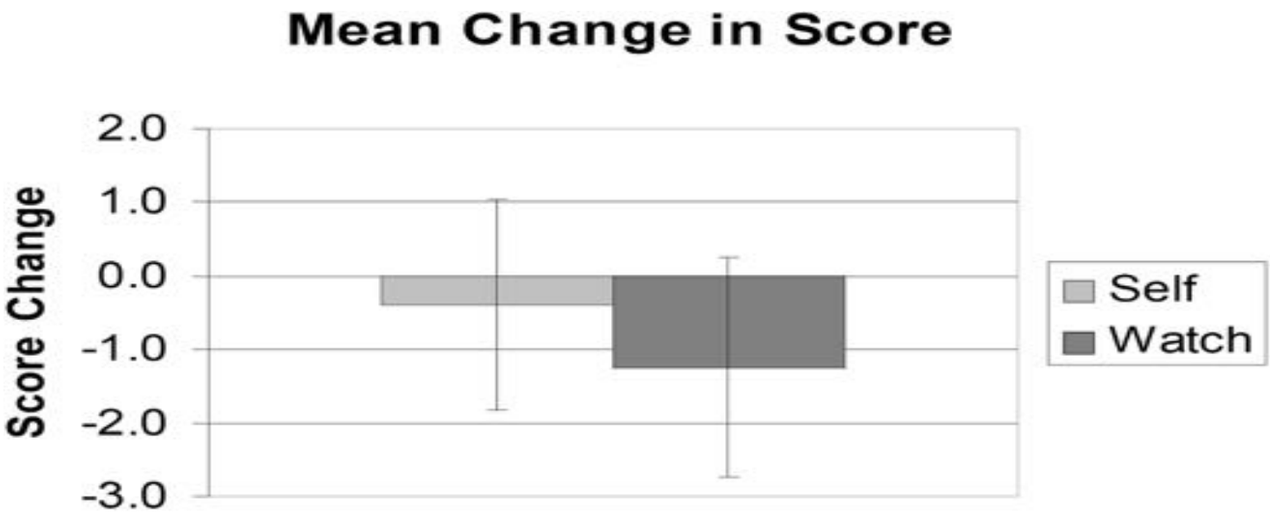
Discussion

The results of the present study indicate that 3- to 5-year-old children do not readily learn the effect of the tubes on the ball's trajectory within 20 trials. Since there was no significant difference between the pre-test and test scores, this indicates that no change occurred in the participants' ability to successfully search for the ball. However, the participants consistently searched correctly at a rate higher than chance, which shows that they do have some rudimentary understanding of the mechanism by which the tubes constrain the movement of the ball.

Since the performance of the children in the "watch" group did not differ significantly from that of the "self" group, there is no support for the hypothesis that observing the experimenter perform the task, including both correct and incorrect responses, caused an improvement in learning.

The frequency with which the search errors were made in the gravity location indicates that children are perseverating on the gravity rule. This could be due to a failure to inhibit the rule that dropped objects fall toward the ground with a straight trajectory. Once children have this knowledge, it may interfere with the acquisition of conflicting information. Children sometimes show difficulty switching rules or inhibiting information in a variety of tasks, and this may play a major role in their perseveration on the gravity error.[6]

Figure 2: Mean change in scores for the Self and Watch groups as measured by subtracting the number of correct searches on the 5 pre-test trails from the number of correct searches from the 5 test trails. Error bars represent standard deviation.



Participants searched correctly on the first attempt 50% of the time in the gravity location on 43% of trials and in the irrelevant location on 7% of trials. Of the errors made, the 86% made in the gravity location is well above the 50% chance between gravity and irrelevant location search errors. Each group showed a slight drop in the mean percentage of gravity errors from the pre-test to the test. In the "self" group, 100% of the errors made in the pre-test, and 91.67% of the errors made in the test were gravity errors. In the "watch" group, 88.89% of the pre-test errors, and 71.43% of the test errors were in the gravity location. Neither of these drops in error rates was statistically significant.

However, the fact that the participants show slight, though insignificant, declines in their overall errors and in the percentage of gravity errors may indicate that they are changing their search strategy as they gain experience. If children perceive that their initial hypothesis is wrong, they may attempt to formulate a new hypothesis or strategy. If they come to believe that the ball will not appear where they expect it to, they might change their search pattern, leading to an increase in the search errors made in the irrelevant location.

The decrease in error scores and gravity errors may also be due to the participants' mistrust of the experimenters or the apparatus. Children may have been nervous as a result of the

interaction with an unfamiliar adult and task. Also, children of this age may be suspicious of games played with adults. Some adults tend to employ deception when playing with children to confuse or surprise them; so, any children exposed to this phenomenon outside of the experiment may have believed that the experimenters were tricking them.[1] This belief could have been intensified in any children who made errors in the pre-test, since they might have formed the belief that their search strategy was incorrect without understanding why and so attributed that to deception on the part of the experimenter.

It is also possible that children were distracted, bored, or lost interest in the study over the training and testing session. This would account for low scores overall, but loss of interest would strongly affect the final test scores and the change from pre-test. The fact that other children were engaged in activities throughout the classroom may have been very distracting to the study participants, especially if they were not motivated to attend to the task.

It is not likely that participants' persistent low scores were due solely to the difficulty of the task. The difficulty level was designed to be challenging, but not impossible for this age group. Prior research has shown that children are able to pass this level of task difficulty (Level III) at approximately 44 months of age.[1] Since the mean age of participants in this study was 53 months, this indicates that factors other than task difficulty confounded the children's performance.

Engaging the children in the task with familiar, trusted adults as experimenters and performing the study in a less distracting environment would help to clarify the results of this study. We are currently expanding our sample size to further investigate the correlation between age and performance, and at what age children are typically able to learn the role the tubes play in the constraint of object movement. It is clear, however, that children approaching 5 years of age may still persist in making errors that are consistent with the gravity rule that objects fall in a straight trajectory. Knowing how children form this rule and how they learn variations or subsets of this rule would allow for a more complete understanding of human cognitive development.

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Overexpression of SMURF1 inhibits BMP signaling in chicken articular chondrocytes and murine C3H10T1/2 multipotent mesenchymal cells

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Chondrogenic precursor cells undergo chondrogenesis to differentiate into chondrocytes that will eventually be replaced by bone through endochondral ossification and articular chondrocytes that remain as cartilage until the onset of OA. In endochondral ossification, chondrocytes stop dividing and increase in volume. They become hypertrophic chondrocytes that alter the matrix by adding type-X collagen and more fibronectin, enabling the matrix to be mineralized. The chondrocytes eventually die by apoptosis, resulting in the loss of the cartilaginous matrix and replacement with bone tissue.

Similarly in OA, seized maturation of articular chondrocytes switches to proliferation and hypertrophy. Observable changes occur in the matrix as well. The cells proliferate and express type-X collagen and alkaline phosphatase (Buckwalter, J. A. et al, 1997) and lay down more matrix (Aigner, T. et al. 1997; Martel-Pelletier, J. et al. 1999), causing. the cells to produce more enzymes to destroy the matrix, such as collagenases and aggrecanases, so the articular surface is worn away (Buckwalter, J. A. et al. 1997; Goldring, M. B. 2000; Martel-Pelletier, J. et al. 1999; Martel-Pelletier, J. et al. 2003).

BMP, which is a potent inducer of chondrocyte maturation, binds dimeric BMP receptor, which then phosphorylates Smad1, 5, and 8 (Miyazono, K. 2000). The phosphorylated Smad proteins form a complex with Smad 4 which acts as a transcription factor (Massague, J. et al. 1997). There is a balance between TGF-_ and BMP signaling in healthy cartilage. During OA, articular chondrocytes exhibit a decrease in TGF-_ signaling and an increase in BMP signaling. These cells can be triggered to cease maturation by TGF-_ (Ballock, R. T. et al. 1993; Ferguson, C. M. et al. 2000; O'Keefe, R. J. et al. 1998) and to increase the rate of maturation by BMP-2 (Grimsrud, C. D. et al. 2001; Sailor, L. Z. et al. 1996), indicating that the balance between these pathways may induce the emergence of OA pathogenesis. It has been speculated that the decrease in TGF-_ signaling leaves more Smad 4 available for complex formation in the BMP signaling pathway. This disrupts the balance between TGF-_ and BMP and induces hypertrophic maturation of the cells. SMURF1 is an E3 ubiquitin ligase that marks Smad1, 5, and 8 for degradation by the proteosome (Zhu, H. et al. 1999).

We over expressed SMURF1 in the murine chondrocyte precursor line C3H10T1/2 and chick articular chondrocytes to stimulate endogenous negative regulation of the BMP pathway. Our data indicates that this leads to strong inhibition of BMP in transfected C3H10T1/2 cells, while the results in chick ACs were not significant. Correlating the results from our animal models and the molecular events of OA, we predict that this over expression of SMURF1 will effectively stop AC hypertrophic maturation and apoptosis, possibly providing a novel form of therapy to alleviate OA symptoms.

Materials and methods

Molecular Reagents

BMP-2 was a gift from The Genetics Institute (Cambridge, MA). The 12XSBE-OC-luc plasmid and SV40-renilla luciferase plasmid were acquired from Dr. P. Luvalle (University of Calgary). The flag-tagged SMURF1 mammalian expression plasmid with the CMV promoter was provided by Dr. J. Wrana (University of Toronto). The empty vector CMV plasmid was sub-cloned in the Center for Musculoskeletal Research at the University of Rochester by Dr. Q. Wu.

Cell models and culture

Murine C3H10T1/2 cells were obtained from American Type Culture Collection. These cells were maintained in cell culture flasks and used for experiments between P4 and P12. Medium was changed every 48 hours, and the cells were passaged at ~90% confluency with trypsin-EDTA (Invitrogen) and fresh growth medium containing Hi-glucose DMEM (Invitrogen) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 0.1% Penicillin-Streptomycin (Invitrogen), and 50 _g/ml ascorbic acid (Sigma).

Primary articular chondrocytes were isolated from 6 to 7-week-old chickens, which were sacrificed by CO2 and cervically dislocated. The legs were dissected, removing the articular cartilage that was digested using trypsin, hyalurinodase, and collagenase. Then the ACs were plated in 12-well plates at 1.5x105 cells per well with 1 ml growth medium consisting

of Hi-glucose Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 0.1% Penicillin-Streptomycin (Invitrogen), and 50 _g/ml ascorbic acid (Sigma) per well and incubated for 24 hours at 37°C with 5% CO2 to allow cellular adherence to the plate surface. Medium was changed prior to transfection.

Transfection

C3H10T1/2: These cells were plated in 6-well plates at 1x105 cells per well and transfected with the same plasmids using FuGENE 6 reagent (Roche Diagnostics). The transfection ratio of FuGENE 6 to DNA was optimized for these cells following Roche's protocol for optimization of cells in suspension. High density micromass cultures were formed by plating the cells at 1x105 cells per 10 _l media in 24-well plates. The micromasses were incubated for 1 hour to facilitate condensation step of chondrogenesis, then gently flushed with medium (Denker et al., 1999) and transfected 6 hours later.

Chick ACs: 24 hours after isolation, the ACs were transfected with CMV-SMURF1 or CMV-empty vector, 12xSBE-OC-Luc, and SV40-renilla luciferase DNA vectors by lipofection using a Superfect transfection reagent (Qiagen) for 2.5 hours. Then they were washed with 1X Phosphate buffer saline (PBS) (Gibco), the growth medium was replaced, and the cells were incubated for 24 hours. Select cells were treated overnight with BMP-2 reconstituted at 100 ng/_l, then harvested for Luciferase assay.

DNA

Plasmid DNA	Lab stock concentration
CMV-empty vector	0.3 _g/_l
CMV-SMURF1	1.539 _g/_l
12xSBE-OC-Luc	0.803 _g/_l
SV40-renilla luciferase	10 ng/_l

GFP

CMV-SMURF1 was transfected into cells to over express SMURF1 in the cells that incorporate the plasmid. CMV-empty vector was used for the controls. 12xSBE-OC-Luc is a construct of 12 repeats of Smad binding elements and osteocalcin (a promoter that is very responsive to BMP) tagged to the firefly luciferase gene that shows activity when there is BMP signaling. SV40-renilla luciferase was used as a transfection efficiency control. SV40 is constitutively active.

Controls

The controls were transfected with the empty vector.

The negative control was not treated with BMP-2. Cells with SMURF1 plasmid but lacking BMP-2 were not expected to activate the pathway involving Smad 1, 5, and 8 because no BMP-2 ligand was available. This controls for the effect of SMURF1 over expression in cells that were treated with BMP-2, which were expected to show strong inhibition.

The positive control served as a basis for comparison. Cells transfected with the empty vector were expected to show high luciferase activity in response to BMP-2.

Luciferase Assay

Luciferase assay was conducted using the Dual-Luciferase, Reporter Assay System (Promega). 24 hours after BMP-2 treatment, cells were harvested by the following method:

Growth medium rinsed out with 1X PBS, 100 _l passive lysis buffer (Promega), was added to each well, and the plates were placed on the shaker at room temperature for 45 minutes to allow the complete lysis of the cells. The samples were stored at -80°C for 3 days maximum until the assay date, at which they were allowed to thaw at room temperature. The assay was run according to the protocol provided by the kit using an Optocomp1 luminometer (MGM Instruments, Inc.). Readings were taken for 10 seconds per sample.

GFP Measurement

C3H10T1/2 cells were transfected with GFP as well as the other plasmids to optimize conditions. Optimization was designed according to Roche's suggestions. These cells were plated at a concentration of 7.5x104 in 12-well plates. The DNA was given 15 minutes to form the complex for incorporation into the cells as recommended by Roche. This FuGENE 6-DNA complex was added to the wells in a drop-wise manner to increase overall transfection efficiency. The cells were incubated for 48 hours and pictures were taken at 20x using an AxioCam MR (Zeiss) camera with an exposure time of 47.211 seconds. AxioVision LE was used to improve visualization, by applying the same parameters to all images.

Statistics

Results of all quantitative assays were analyzed via ANOVA with sample sizes between 3 and 6 and calculated p-values of less than 0.05 being taken as indicative of significant differences between groups.

Results

Six trials for AC transfection were run. Transfections were successful as indicated by high level of activity in the Luciferase assay data (read-out showed numbers above 1000). However, 12xSBE-OC-Luc did not consistently show a good response to BMP in the control cells.

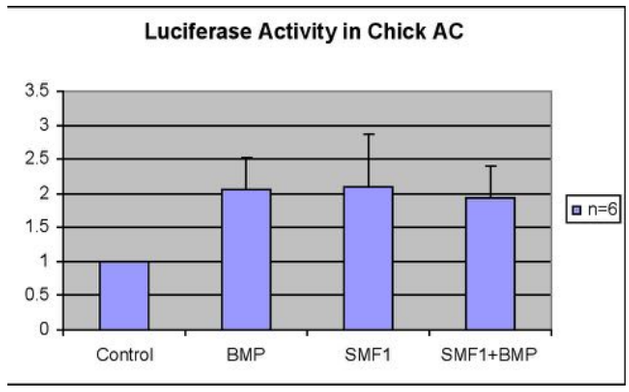


Figure 1. Articular chondrocyte BMP signaling was induced by BMP-2 but was not inhibited by Smurf1. ACs were plated at 1.5x105 cells per well in 12-well plates. Control cells were transfected with CMV-empty vector plasmid. *When these cells were treated with BMP-2, they showed high levels of activity. Cells transfected with Smurf1 were treated with BMP-2 to test for inhibition. All cells were harvested for Luciferase assay following the procedure in Materials and Methods (p-value < 0.05, N=6).

The transfection of C3H10T1/2, using Superfect as the

lipofection reagent, was unsuccessful as indicated by luciferase read-out values below 1000. Superfect may have been toxic to the cells, so FuGENE 6 was used as an alternate reagent. The optimal transfection efficiency was achieved with a Transfection ratio (reagent:DNA) of 3:2. Transfection ratios of 6:1 and 3:1 yielded poor results compared to 3:2. 6:1 gave only 10% of the efficiency of 3:2, while 3:1 gave approximately 20% of the transfection efficiency of 3:2.

Initially, transfection of C3H10T1/2 was 24 hours prior to plating in micromass. They were fed 100 ng/ml BMP-2 after 24 hours of incubation, and then harvested for the Luciferase assay 24 hours after treatment. This transfection was ineffective as indicated by the low luciferase activity read-out. One possible reason is that the cells may have needed more time to recover from lipofection before being plated in micromass cultures.

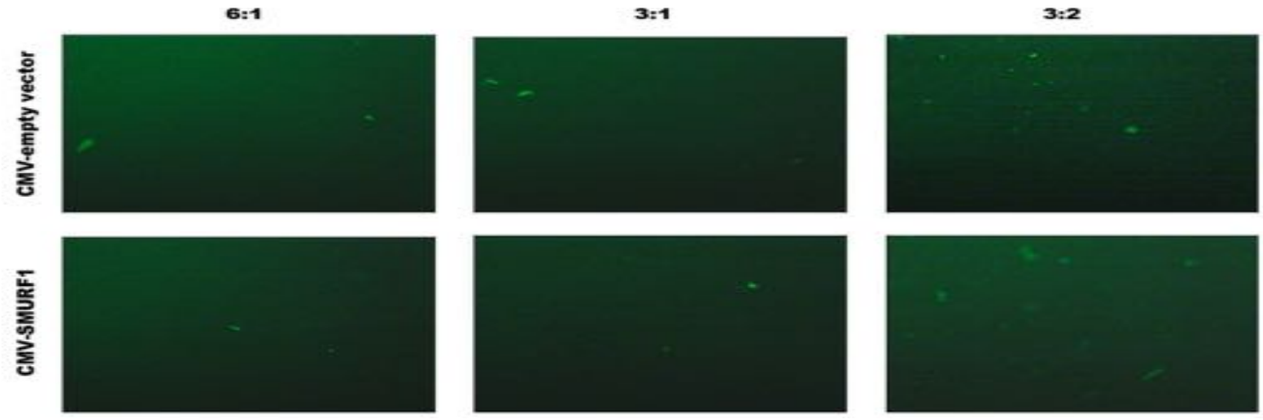
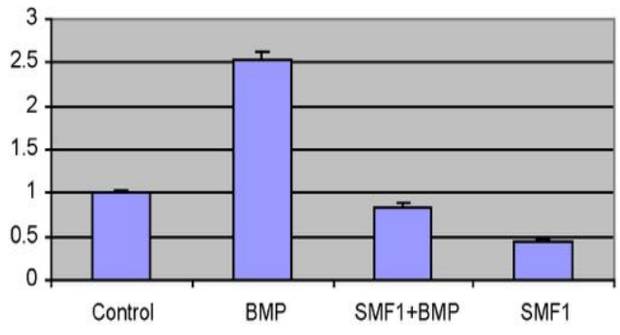


Figure 2. Results from suspension transfection optimization of C3H10T1/2 cells using FuGENE 6 showed that maximum transfection efficiency was obtained from a 3:2 ratio of FuGENE 6 to DNA. Ratio of FuGENE 6 to DNA indicated across the top of the figure. Cells were plated as with AC experiment. They were transfected with the same plasmids in addition to GFP.

The 3:2 ratio was used for transfection of C3H10T1/2 in micromass and cells plated in monolayer in 6-well plates. The cells were transfected 24 hours prior to formation of micromass due to post-trypsinization cell sensitivity. All cells were treated with 100 ng/ml BMP-2 24 hours after transfection and harvested for Luciferase assay 24 hours after treatment. Cells over expressing SMURF1 and plated in 6-well plates showed robust inhibition of BMP signaling when treated with BMP-2. Additionally, the positive control indicated that 12xSBE-OC-Luc was highly responsive to BMP-2.

Figure 3. 10T1/2 BMP signaling was induced by BMP-2. Signaling was significantly inhibited in Smurf1-transfected cells. The cells were plated at 1.5x105 cells per well in 12-well plates. Cells designated for treatment with BMP-2 were treated with 100 ng/ml BMP-2, incubated for 24 hours, and harvested for Luciferase assay as described in Materials and Methods. *The promoter was highly responsive to BMP. **There was robust inhibition of



Discussion

The pattern found in ACs may stem from 3 possibilities:

1) Basal BMP signaling in ACs may be low to begin with. Therefore, the inhibition may be too small in scale to detect with the Luciferase Assay. Determination of basal BMP signaling will provide useful information for calibration or choice of a more sensitive assay method.

2) Transfection in ACs was not yet optimized for these particular plasmids with Superfect. Although the Luciferase assay readings show transfection levels were sufficient to obtain a reading, this may not be optimum since only a few cells were actually over expressing SMURF1.

3) The final possibility is that SMURF1 does not act as a negative regulator of BMP signaling in ACs, although over expression of this inhibitor has had significant effects on other BMP-responsive tissues such as osteoblasts (Zhao, M. et al 2004) and committed myogenic precursor cells (Ying, S. X. et al. 2003).

Transfection optimization of C3H10T1/2 indicated that a 3:2 ratio of FuGENE 6:DNA was the optimum condition. In the positive control, 12xSBE-OC-Luc was highly responsive to BMP as indicated by the Luciferase assay. C3H10T1/2 cells transfected with SMURF1 and treated with BMP-2 showed strong inhibition while the BMP-2 signal was effectively blocked by overexpressed SMURF1 ubiquitin ligase. This verifies that BMP signaling in C3H10T1/2 cells can be blocked by SMURF1, which targets Smad proteins involved in the pathway. C3H10T1/2 represents the effect of BMP inhibition in chondrocyte precursor cells.

Future work involves verifying that over expression of SMURF1 in articular chondrocytes (ACs) results in significant negative regulation of BMP signaling as seen in C3H10T1/2. A

complete understanding of these signaling pathways involved in the disease process would form the basis of future experiments designed to test the idea that BMP signaling inhibition could represent a potential therapeutic approach in the treatment of OA.

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On the Road: The Rhetorical Action of Billboards

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As landscape theorist J. B. Jackson emphasized in *Landscape* magazine: "Never before had there been so total and dramatic a transformation of a portion of the American landscape, so sudden an evolution in habits, nor such a flowering of popular architecture" as demonstrated by the introduction of the automobile to our society. With the increased mobility of this invention, Americans began experiencing life in ways they never had before. The pace quickened as further technological advances in media reduced the attention span and evolved literacy of the public eye. These developments were recognized and capitalized by business entrepreneurs of industry, particularly outdoor advertising. Public communications in the form of billboards are an American invention that has displayed levels of versatility through creative expression that only a free-market system could harbor. Through a rhetorical perspective, billboards are cultural artifacts that allude to the American consciousness in context with history. Though there continues to be much controversy over the responsibilities for use of roadside space, the medium remains a prominent aspect of the American landscape. Even while situated within the high-speed internet age, billboards continue to evolve through and directly reflect the cultural fluctuations that implement them.

Roadside Evolution

An overview of historic events and the parallels between a morphing culture and vernacular qualities of public space preserve an interesting narrative. I will examine society's progress while keeping in mind Robert Bales' concept of "fantasy themes" and Ernest Bormann's application of these themes to small groups in the realm of mass communication. To provide insight into how messages were passed from small groups to contexts such as the media and public address, Bormann noted that Bales' work implied "the dynamic process of group fantasizing" which was an extension of individual fantasies.[1] As the American consciousness evolved under a capitalist system, alternatives to traditional values emerged in contrast to a source of authority attempting to govern and establish order. Such forces have influenced social climate and appeared as artifacts in forms of architectural design, public policy and aesthetics. These cultural artifacts will be highlighted in the evolution of public space as demonstrated by the growth of cities and business at the turn of the twentieth century.

In addition to the rapid development of urban downtowns and Main Streets, the introduction of the automobile has drastically shaped the vernacular space along the road. In particular, drivers and passengers became a profitable audience and the road, a space of freedom which I will more thoroughly examine later. With the recognition of this opportunity, public roadside space was quickly implemented as a medium for mass communication.

Ever since lithography was invented in 1795, the potential for poster advertising has existed as an effective medium for persuading viewers. Advertising comes from the two Latin words, "ad," meaning "toward," and "vertere," meaning "to turn." Therefore, the objective of advertising is defined: to *turn* the attention of any given market buyers *toward* a product, service, idea or personality. As a tool and technique, advertising is a vital part of marketing, an overwhelming force within a free-market economy system and in effect, has created an industry of itself. Advertisers "buy" an audience by most effectively communicating to consumers through an appropriate medium. Since very few people are exposed to advertisements during work and none during sleep, advertisers must communicate to the audience during their leisure time or while driving. The unique benefit of roadside advertising minimizes the work of circulating a message within the market of buyers by instead, allowing the market to circulate around a message.[2]

Outdoor advertising can be divided into two major classifications—on-premise and off-premise—within which exist more categories ranging from small store signs to huge electric billboards. The on-premise aspect of outdoor advertising is most commonly referred to as the sign industry. Such signs are used to physically identify roadside businesses and draw attention to the products and services that are offered by that location. Off-premise advertising allowed opportunities for businesses to lure consumers and generate anticipation as advertisements were used as tools for directing the market. Many retailers chose to escape the chaos, leaving crowded downtowns behind and embracing the freedom of the open road spaces where regulations were less stringent.

The concept of constructing a special structure dedicated for poster advertisement was a seemingly modest development but a revolutionary one as the standardization allowed the

Figure 1: Henry Reuterdahl spent more than a decade with the US Navy as an artist-correspondent to produce this 1917 recruitment poster (31" x 43"). *Reprinted in American Billboard: 100 Years, 25.*

advertisement to be placed in a landscape with a certain authority over the medium. Although the British history of leasing space for posters and billposting as an occupation predates American methods by nearly a decade, the United States was the first to implement a standardized, freestanding billboard structure solely dedicated to these purposes. Britain did not develop regulated structures until the early twentieth century, making the bold, framed figure of the billboard a true American invention.[3] A billboard is any large poster mounted in a public place, whether for advertising, political propaganda, or even decoration. Most common billboards are outside structures that are freestanding and often lit for visibility at night. As described by the curators of a 1999 Massachusetts Museum of Contemporary Art (MASS MoCA) retrospective exhibition highlighting artist's billboards, the poster is an "active medium for the selling of ideas, the motivation of consumers and the expression of artistic and design ideals."[4]

Jared Bell created the first forms of billboard advertisement in New York by placing multiple nine by six foot posters in an arrangement to produce a larger image. As the printing press technology progressed, larger sheets were produced and soon the limitations in size were only constrained by the structure to which they were ultimately fastened. During the mid-nineteenth-century, circus and theater groups were natural clients for posting ads in this way. Almost any surface could be made suitable for poster display; fences, building walls, windows, rocks and especially the train systems when the NYC elevated railway opened in 1867. City centers and even larger outskirt towns quickly became a clutter of crudely posted signs, flapping and fading in the streets. With all this potential in plain sight, advertisers began to capitalize on the benefits of organized and regulated marketing systems.

By 1890, posting services in Chicago, New York and St. Louis began reflecting more awareness of their responsibilities when occupying public space. The members of the International Bill Posters' Association formed an associated group for the United States and Canada lead by founding President Edward A. Stahlbrody of Rochester, New York. Stahlbrody outlined purposes to promote a greater understanding of the poster medium, to organize and coordinate services all while addressing ethical concerns.[3] As businesses within town and cities began to develop across the nation, the technology and competition of the outdoor advertising industry progressed respectively.

Main Street became a site of a "full-scale visual tug-of-war" between the traditional structures of the past and the new commercial presentations of modern design.[5] Builders of new downtown structures included decorative details in brickwork and facades but also were attentive to including storefront windows for advertisement. Designers began to collaborate with sign makers for the most effective use of roadside space and the attraction of customers. The 1879 invention of electrical light was welcomed with enthusiasm as cities soon became a dramatic and sparkling sight of illuminated signs. As author John C. Van Dyke describes, "letterings, patternings, arabesques, figures

Figure 2: Designed by Maxfield Parrish in 1917, this twenty-four sheet ad promotes Fisk Rubber Company for spare tires—then referred to as "shoes." *Reprinted in American Billboard: 100 Years, 31.*

Figure 3: This 1965 Ford billboard encourages a suburban family to own more than one vehicle; father drives to work while mother drives to the grocery store.
Reprinted in American Billboard: 100 Years, 97.

outlined by small electric globes...sometimes blinking…All told, the glitter and glare of these signs make up a bewildering and…brilliant sight."[5]

Such illuminations soon became overwhelming and littered the night sky. Architects, contractors, city planners and citizens concerned with the degenerating conditions and appearance of these urban areas recognized a need for reform. In the early 1900s, Daniel Burnham became a key figure of the City Beautification Movement by suggesting, "the landscape [would] complement the burgeoning reforms in other areas of society" referring to the poverty and crime that was plaguing crowded urban centers.[6] The City Beautiful leaders were mostly upper-middle class, white, males who believed that an emphasis on creating a beautiful city would in turn inspire its inhabitants to moral and civic virtue. This discourse became more and more complicated as conditions in the country's urban areas continued to fluctuate within a web of interconnected factors, specifically transportation and architecture in relation to outdoor advertising. Arguments of the movement more or less concluded that billboards should be recognized as a separate class of structures requiring distinct regulations of their own.[4] There were many opposing opinions around this verdict, many of which remain issues that continue to be of active debate.

Billboards as Rhetorical Artifacts

Advertising practice encouraged self-regulation and organization, however, the extent to which the billboard's innovated opportunity was utilized within culture multiplied

Figure 4a., 4b: Tri-vision
Image obtained from: <http://www.fhwa.dot.gov/realestate/elecbbrd/appxa.htm>, <http://www.infogoldmedia.com/billboards.htm>

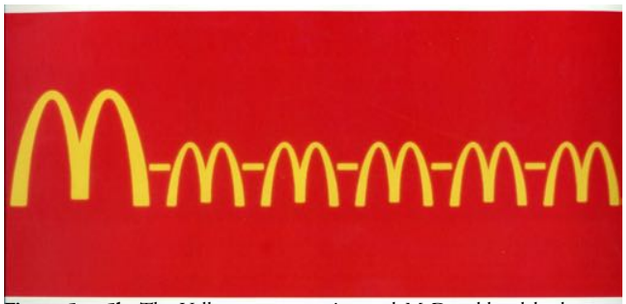

Figure 5a., 5b: The Volkswagen campaign and McDonalds ad both present simple designs with reduced text that are most effective for communicating memorable messages.
Reprinted in American Billboard: 100 Years, 125, 158.

drastically by other occurrences in society. This amplification relates back to the themes of personal fantasies that become engaged on a larger level. As models of tradition are established, they can often operate as powerful tools of communication and reinforcement. Relating to Bormann's "symbolic convergence theory," the belief that communication creates reality generates an understanding of rhetorical visions. Within society, people try to make sense out of what is happening. To do this, they create and use symbols to frame and define their understanding of reality. Bormann's theory suggests that these symbols exist throughout the process of communication and by using them to make sense out of one's social and physical environments, people become linked together under a powerful network of mass communication. In the case of outdoor advertising, this understanding of convergence within communication will allow for amplification of a controlled perception. As Bormann explains;

> *The sharing of fantasies within a group of community establishes the assumptive system portrayed in the common rhetorical vision and contributes to the special theory associated with the community's communication style.*[1]

Through the amplification that the billboard industry experienced, a strategy of influence becomes evident from a rhetorical perspective of billboards as cultural artifacts. This perspective examines what influences and persuasions are conveyed to the public in billboards during various historic moments as well as the reasons for such influences. Rhetoric is social and public, as it addresses others, and therefore uses the values accepted and affirmed by a subculture or culture as reasons. In this way, rhetoric is tied to social values, and reflects the social norms of particular times and places.[7]

As the United States became involved in World War I, billboard content began reflecting patriotism and messages of pride and support for the services. Nationwide campaigns were organized to promote the Navy and artists were gathered to design the billboards. Educators got involved and issued a "Call to Service" that encouraged "art to exist for the sake of the republic" by funneling resources at the Art Institutes and New York School of Fine and Applied Art to make posters for the Army and National Guard.[3] Another war mobilization effort in 1917 highlighted the Liberty Loan drives, a project that over the course of three years, filled $1.5 million worth of donated outdoor advertising space. After the war was won, the positive involvement that the billboard industry had demonstrated to the country was rewarded with a level of acceptability for long-term industry objectives.

During the 1920s, the American economy flourished and the advertising industry mirrored this explosion of consumption with immense marketing campaigns. Roadside business began to boom as the nation embraced a release of a youthful, carefree, rebellious attitude that identified with the jazz age.[4] In 1921, the Federal Highway Act was passed to enable economic growth to continue with an essential addition of national road systems. This again boosted the outdoor advertising industry creating miles and miles of new billboard opportunities. All sorts of products were promoted to passing drivers including the essentials of extended road trips—batteries, tires and food. In 1925, the first instances of conglomeration occurred as the General Outdoor Advertising Company (GOA) became a giant of the East with the best in-house artists. In addition, the Outdoor Advertising Association of America (OAAA) formed and remains to this day, a standard in leadership and service to protect, unite and advance a responsible outdoor advertising industry that is committed to serving the needs of advertisers, consumers, and the public. These alliances created a more efficient network linking merchandisers, advertisers and specialized information. Growth in tourism experienced by restaurants and hotels also resulted from the merge. These facilities encouraged road travel by accommodating drivers and passengers on their journeys and luring them to various tourist attractions that were designed as milestone destinations along the road.

This ambiance of the roaring twenties was short lived, however, as the next decade left America in a deep depression with a president whose desires for normalcy recoiled society back into a tradition of home and family. The home became a symbol of cultural success as homeowners were respected within communities. At a 1931 Home Building Conference, Herbert Hoover described the home as a "castle in all that exquisite sentiment which it surrounds with the sweetness of family life."[5] As a result, the roadside merchants adopted domestic imagery of the home in advertisements. Businesses embraced a homey atmosphere with green lawns and white fences that welcomed customers from the road. Construction of new bungalows and more billboard frames helped to stimulate the lumber industries and create jobs as well.

During World War II, the billboard content shifted to reflect propaganda by addressing every citizen as a combatant in a war of production that sold the idea of the factory and home as arenas of war. Popular imagery included Uncle Sam and a prominent American flag as the government publicity campaigns created a base of national solidarity and support. The advertising industry helped encourage these national

Figure 6: A 1962 ad for Grape Nuts emphasizes the trends of diet, health, fitness and body image that became more desirable into the seventies. *Reprinted in American Billboard: 100 Years, 137.*

icons by linking them to consumable products. War billboards represent the earliest forms of artist billboards and established a precedent for public service addressing political and social issues.

Coinciding with a shift in demographics following the Depression and World War II, suburban masses began growing as GI Bills allowed more people to buy houses. The government reacted to the necessity of fueling industry after the war by stimulating production through consumption. The image of the home resurfaced to encourage sales to housewives who maintain the home environment by embracing this domestic image. In addition to this introduction of numerous new products, the marketplace experienced another influx of automobiles that were necessary for allowing the transport of goods from shopping centers to home.

The existing roadways of downtowns and country routes suffered from "autoclerosous." These crowded conditions, not uncommon from the congested cities became a major concern during the Cold War as fears of nuclear war loomed over American culture. In 1947, the government passed the National Interstate Defense Act that called for 37,000 miles of new road to provide a substantial route for evacuation, if need be. Of course, with these new roads came new opportunities for billboards—this time soothing and reassuring the American public during the threats of an atomic age.

An overview of the first half of the twentieth century displays the integration of societal beliefs with authoritative action around issues of public space. The symbolic representation that appears in vernacular design, particularly billboards, became more refined as the creative energies of the second half of the century surfaced. An emerging counterculture opened a realm of alternative approaches to life. The advancement in goals of freedom, moral excellence and liberty were agitated and polarized towards a progressive American ideology. At the same time, advancements in aesthetics and use of new technology amplified the effectiveness of symbolic language, which I will examine in the next section. This not only increased competition in the spectacular presentations that were created for billboards, but also complicated the rhetorical discourse around the medium.

Aesthetics of Billboards

In an essay by Kenneth Burke entitled "The Philosophy of Literary Form," he argues that effects of art and its appeal are always historically contextualized because "aesthetic values are intermingled with ethical values—and the ethical is the basis of the practical."[1] Billboards present a manifestation of this aesthetic sphere as they have served as an evolutionary medium that has allowed various phases of this aesthetic value to be displayed. Although billboards are ephemeral, documentation of their imagery has been cataloged by certain organizations, most thoroughly by OAAA in an online database. The sequences of billboards during the later half of the twentieth century display the upheaval of social structures that occurred. As an artifact, the billboard demonstrated the progress of art and technology in its refinements of style and design. Of course, these shifts in social and aesthetic structure towards a modern culture are most notably reflected in the actual advertisement styles. The cultural resonance in these billboards contributes to their rhetorical significance as they help to meditate conditions of acceptance and response. As Burke further states;

> *Art is the strategic naming of a situation. It singles out a pattern of experience that is sufficiently representative of our social structure, that recurs...for people to need a word for it and to adopt an attitude towards it.*[1]

The portrayal of these attitudes through the aesthetic qualities of billboards maps evolution of style, culture, technology and the media literacy of America.

The cutout image, which extended outside the standard billboard frame, emerged as one of the most far-reaching trends of the 1950s.[3] In addition, innovations such as reflective tape, mechanical parts and back lighting were "surprise elements"

when viewed at night. These additions later developed into the "full bleed" board that allowed printing on the blanking paper as well as the poster thereby displaying an image without a frame at all.[2]

Despite economic concerns, the creative revolution on billboards continued as technology provided new techniques and cultures of art produced new imagery. In fact, the relationships between these developments left their own trademark style through each consecutive decade. For example, during the Great Depression the teardrop shape was a favorite motif that was duplicated in automobiles, appliances and furniture.[8] This streamline identity put an emphasis of photorealism in billboard illustration and though photographs were dominating the magazine industry, they did not appear on billboards until years later.[5] Eventually graphic design became prevalent and with the introduction of silkscreen and plastics. This ultimately led to computer painting on vinyl which was advanced by Metromedia Technologies and Computer Image Systems.

Of course, technological advancements did not always work in favor of the billboards. With radio and television opening new arenas for an advertising market, the competition grew. In most cities and towns, there were often a couple outdoor advertising plants. These plants controlled the majority of public space by selling units or "showings," in different packages of any national, regional or individual markets. Similar to how broadcast would sell minutes or hours of airtime, outdoor advertisers would sell showings of relative numbers that included an amount of prints installed within the market. A market is divided into poster zones of approximate percentages of principal streets with 80 to 90 percent of traffic leading to business areas. Of course, these packages are mere outlines, as many showings are often tailored to suite the specific needs of a particular client. Showings also varied in value as determined by the creative features any billboards included. A popular variation was the "tri-vision" or "multi-vision" that divided the face of a billboard into individual vertical strips of triangle panels that rotated allowing more than one message to be shown on the same panel.[2]

The automobile was an essential element for billboard expansion as it coincided with the postwar production and rapidly expanding economy. The automobile allowed the suburban families to feel strong and content because of what could be provided through this high standard of American lifestyle. In 1954, another expansion of the national highways network was issued by the governments Federal Interstate Highway Act except this time, the plan included restrictions for billboard sites. This was met by extensive lobbying from the billboard industry over their concern for existing structures that were adjacent to the proposed interstates. Controversy around the aesthetics of community and scenic interests heightened through the sixties as more and more funds were diverted towards these debates which were more aptly described as "public education."[3]

With the emergence of a counterculture during the 1960s, many advertisers shifted their focus to a new, youthful market of easy persuasion. A surprisingly effective merge of the copywriting and art direction into one "creative department" reorganized the principles of campaigns to "simplify… dramatize…make crystal clear and memorable."[3] Television had radically changed American's attention span and as a result, the constant stimulation from such technology and freedom of mobility through vehicles pushed the pace of society to grow faster and faster. This was beneficial to billboard literacy as there exists a short period of time as a viewer approaches a sign to be able to read it. The effects of minimalist ideology are seen in billboards that use few words and instead rely on imagery of concepts alone. For many, a shift from the nuclear family to value of oneself also opened a new approach in advertising as products were targeted to individuals seen especially in food advertisements. Great emphasis was put on the human body encouraging love and pleasure for oneself and as a result, iconic human models proved successful gimmicks for product presentation. Humor and directness contributed to a phenomenon of designs that were distancing themselves from the banality and fantasy of the 1950s and intentionally blurring lines between advertising and graphic entertainment—all of this, a stimulus to pop artists.

Emergence of Road Culture

The development of a political and class consciousness reached a forte during the 1960s. An urgent reexamination targeted many ideas and expectations that were previously held at high national standard. Political and social revolts of the sixties were revolts against values that began questionable consideration during the Cold War atmosphere of the 1950s, long before Kennedy's assassination, political scandals and the Vietnam War erupted into the consciousness of the American people. After 1969, Americans entered a new world of expansive government that agitated the cultural divide throughout the 1970s.[9]

Though the conservative traditions were eventually reaffirmed by Reagan's era, subcultures of alternative perception peeled away from popular consensus during this period and began to practice new approaches, rejecting a culture of suspicion and control. A focus on personal freedom and individual liberation introduced new symbols and ideologies of American lifestyle such as escapism. As beatnik author Jack Kerouac simply states; "the road is life" and presents the metaphysical significance of the road as culture during this period in history.[1] People used their cars as extensions of themselves and enjoyed the liberation that driving an open road offered in contrast to the spiritual poverty of traditional American life. The road becomes a symbol of mobility that reminds Americans of their true wealth and potential. As Kerouac's 1955 novel, *On the Road* presents, the road is a path of transcendent experience,

Figure 7: 1975's Miss America recipient, Shirley Cothran, featured in a billboard campaign becomes nationally recognized.
Reprinted in American Billboard: 100 Years, 152.

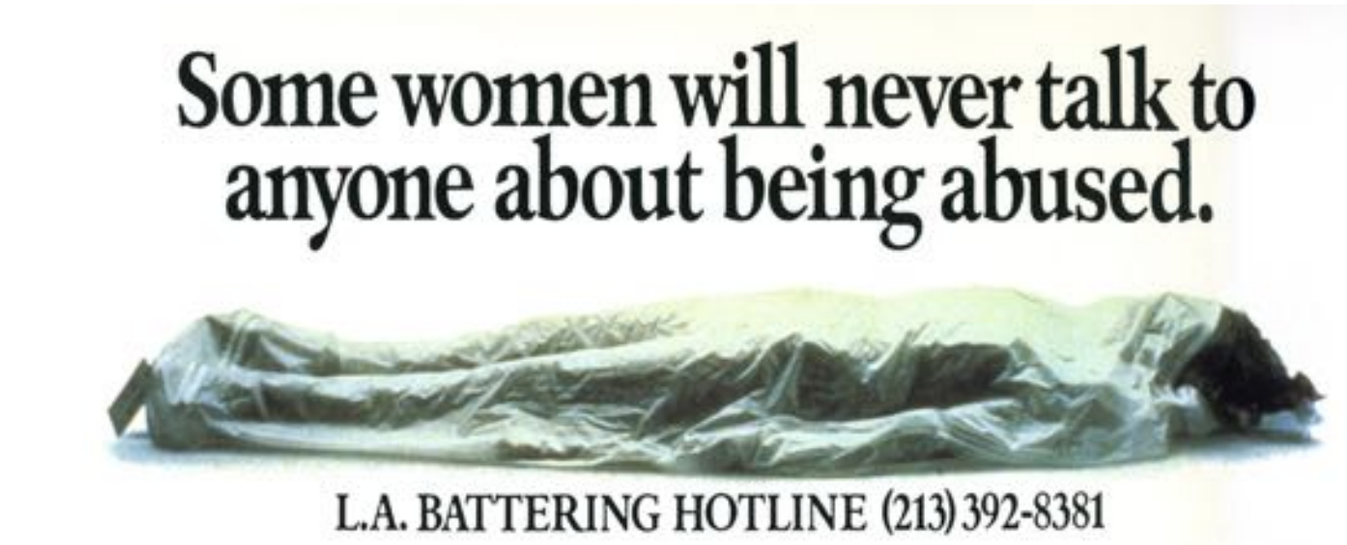

Figure 8a: Public service announcements regarding domestic violence became a common sight during the eighties. The design provokes shock that attempts to reach out to abused women with a strong visual and emotional impact that could encourage victims to seek help through a provided hotline number. Similarly, the literal consequences of drunk driving are displayed on another billboard.

taking travelers away to a fantastic "landscape and mindscape seen from terminal velocity."[1]

Revisiting the excessive signage of Main Street that existed prior to the dawn of the auto age, the success of a business often depended solely on the presentation a sign or billboard that would stand out most to passing eyes. The introduction of the automobile simply amplified the vitality of roadside signage and created an overwhelming influence on the evolution of vernacular space. Competition increased and roadside retailers were forced to create a union between sign and building. Contractors and architects adjusted their designs for drivers and passengers in automobiles as the fast-moving eyes required a different presentation of signage. Downtown became a dense space of traffic movements including vehicles and pedestrians alike. As more and more people began using cars to commute to the downtown area of work or shopping, commercial visuals began to cater to an audience in transit, on the road.

This new perception of landscape and life has drastically influenced and amplified the communication through public vernacular space. Similar to the screen of a television set, the windows of a car framed perceptions of the world at high speeds influencing a faster pace and stressing efficiency. Even though the road provided an escape from suburban boredom, the car soon became a substitute for this domestic space and the whole highway system, in a way, became an entire domestic world in and of itself.[10] People began to eat in their cars, listen to the news or music and even sleep. Hybrid vehicles were designed to merge the comfort of a home with the freedom of mobility—these were the first mobile homes.

There are many conflicting opinions on the topic of automobiles and their effect on our culture's development. Though the outdoor advertising industry has equally contributed to the development of structures that complement roadside architecture, the extent to which these structures have molded new perception and reinforced ideals of fantasy is largely due to the personal freedom's experienced by driving an automobile. The widespread mobility that personal vehicles allowed, has transformed concepts of freedom and self-perception, therefore pushing aesthetics of public space towards a refined language of symbolic communication that was seemingly limitless.

Reform Establishes New Consciousness

The competition and bold progressions of billboards during the 1960s further agitated the debate for regulation and beautification of public space along the road. President Johnson stated:

> *More than any country ours is an automobile society. For most Americans the automobile is a principal instrument of transportation, work, daily activity, recreation and pleasure. By making our roads highways to the enjoyment of nature and beauty we can greatly enrich the life of nearly all of our people in city and countryside alike. The roads themselves must reflect, in location and design, increased respect for natural and social integrity and unity of the landscape and the communities through which they pass.*[11]

With his wife, "Lady Bird," leading the effort to secure these ideals, President Johnson made such issues a priority, and through much delegation, the Highway Beautification Act was passed in 1965. The legislation called for states to control outdoor advertising and junkyards adjacent to Interstate and primary highways. Local sectors were encouraged to authorize landscaping and scenic enhancement within the highway corridor. The junkyard control was the most effective aspects of the act as states took the opportunity to erect fences that would screen rusting cars from sight of the roads and give compensation to junkyard owners for removal, relocation or proper disposal.

The billboard industry felt directly attacked by the regulations imposed under the act, however, most of the concerns regarding limitation were a simple misunderstanding of what the act really proposed. The only signs that were not subject to control under the act were on-premise structures that were located on the property where the advertised business was conducted. Other roadside structures were required to be 660 feet from the edge of the right-of-way in addition to lighting, size and spacing requirements. Such improvements under this

Figure 8b: The literal consequences of drunk driving are displayed on another billboard.

act, however, did not receive federal funding unless the state could pass legislation first—an overlooked detail that made the effort appear ineffective. Most noticeably, changes in standardized tourist signs provided information on food, gas and attractions without overwhelming presentations of brands and images. Highway rest areas were constructed as well as landscape designs that enhanced the natural scenery that roads cut through.[8] These improvements began to bring concepts of a public consciousness to shared spaces along the road. Though it is difficult to override the business agendas of these spaces, some states have been successful in limiting outdoor advertising quite a bit. In fact, Alaska, Hawaii, Vermont and Maine have banned billboards entirely.

In 1971, the Institute of Outdoor Advertising (IOA) was established to develop research, creative ideas, promotion and effective use of the medium, which later merged with OAAA to streamline communication. The same year, tobacco advertising was banned from television and radio, which displaced an enormous amount of this marketing on the billboard industry. According to a 1969 article, the super trade organization of OAAA consisted of plant owners from 90 percent of the medium's facilities.[2] In addition to maintaining liaison with agencies and advertisers, the OAAA published trade journals and quarterly publications that provided reports of the national market as well as new developments for the medium and an occasional design contest. Some of these titles include *The Poster* and *Outdoors Buyer Guide*.

The conglomeration of the industry has continued in addition to acquisitions of computer technology allowing more modern campaigns to be designed. The record industry's use of billboards along Sunset Strip in Los Angeles became famous icons for glamour and the emphasis of logo branding and exploitation of the pun during the eighties have left memorable campaigns.[3] In 1975 the IOA launched a campaign to test the effectiveness of billboard advertising, using the image of newly crowned Miss America, Shirley Cothran. Her name recognition soared after the campaign proving the value of billboard medium and provoking interest in studies on such criteria. Organizations such as the Traffic Audit Bureau's focused primary tasks in determining how many people have the opportunity to see an outdoor advertisement. In 1979, The Traffic Audit Bureau (TAB) hired the firm E.J. Sharsky & Associates to re-evaluate its traffic estimating procedures. It was the first complete review of TAB procedures since its inception in 1934. The findings were published in the book *Counting Cars*, which confirmed the power of billboard medium in presenting ideas through public space to mass culture. The widespread impact of this structure, however, made it very difficult for agreements to be reached on authority over this space.

Embracing Opposition

Put most simply, the problem lies in the gap that exists between what one thinks ought to be and what is; it is the discrepancy between the ideal and the real. Due to the diversity of perceptions, a problem for one person may not be a problem for another. Issues regarding use of public space have been of debate from the earliest inception of the concepts' use. Not everyone has the same beliefs in aesthetic value, and therefore different levels of tolerance are directed at the presentation billboards create.

There have been many instances of opposition to the spread of billboards looming over roadside landscape. Some argue that drivers can become distracted as they drive. Studies indicate that a substantial percentage of this distraction can be classed as 'external-to-vehicle'. Billboards and signs at junctions and on long monotonous roads (such as highways) can function as distractions and constitute a major threat to road safety. It is also likely that lights or billboards on long 'boring' stretches of road can surprise drivers when advertisements suddenly appear, or cause fixation and failure to concentrate on driving. This is because these signs create visual 'clutter' thus making it harder for the driver to perceive traffic lights and other safety signs/devices.

There are many examples of the billboard used as space for public service, which tend to favor less radical imagery and encompass more universal appeal. Ranging from issues of homelessness or domestic violence to AIDS awareness, the billboard can serve a very useful purpose in raising awareness of crucial societal problems. Similar to these messages of public advocacy, independent artists have adopted the medium to send their own messages of persuasion to viewers. Sometimes this takes the form of graffiti but has also been executed through larger projects of collective organization between artist foundations and the advertising industry.

During 1998 in North Adams, the MASS MoCA

Figure 9: In 1992, Ron English's "Cancer Kid" was installed in Jersey City on top of a "Joe" Camel cigarette advertisement. English would hand-paint his designs and then paste them over existing billboards.
Image obtained from: <http://www.graffiti.org/ron_english>

commissioned six artists including Leon Golub and Lothar Baumgarten to create new billboards for its community. Most of the artists acquainted themselves with the area and met residents of who shared their interests of discontent with issues ranging from workers rights to vegetarianism. The project directly addressed to extensive abuse of public space and vernacular landscape that was presented at all entrances to the town. Eyesores include factory buildings, tightly packed worker housing, asphalt steeples, smokestacks and, most of all, billboards. This commercial reality in contrast to the beauty of the Berkshire Valley was examined through the project which embraced these very billboards as spaces to reflect the community's disgruntled voice in addition to a retrospective show that exhibited the work as well as other historic examples of such efforts. The exhibition was funded in part by OAAA and private sponsorship that produced twenty-five works of roadside art scattered across Western Massachusetts. Joseph Thompson, the director at MASS MoCa explained in the accompanying catalog;

> *[Artists] were not only utilizing the vernacular space of billboards, they were also appropriating the visual language and rhetoric of commercial advertising in their work and vice versa. The fluidity and richness of this interchange could only manifest itself in an exhibition of large scale.*[12]

More radical artists or "culture jammers" put their own spin on billboards by repainting imagery that often subverts the intended message. Culture jamming is a form of activism that often adopts the qualities and medium of its target and translates the original message into an alternative meaning. Originally demonstrated in forms of vandalism, culture jamming is a clever approach to protest and civic engagement. Adbusters Media Foundation encourages new social activist movements of the information age, aiming to topple existing power structures and forge a major shift in the way we will live in the 21st century. They publish the magazine *Adbusters* that considers itself dedicated to examining the relationship between human beings and their physical and mental environment. The foundation has also launched numerous campaigns such as the black spot that "rethinks the cool" on sneaker corporations like Nike who employ sweatshop labor. Large black spots are scribbled over the logos of corporate billboards, such as Nike's trademark "swoosh."

Similar to black spot, the graffiti artist, English's M. O., has been pirating billboard space since the eighties by posting his own hand-painted designs over the existing ads. He has created anti-ad campaigns in Texas, New York and New Jersey. For example, Camel's "Old Joe" has often found himself replaced with "The Cancer Kids." Defacing corporate mascots from their top dollar billboards is of course punishable under law. Many artists accept this risk; in fact, the Billboard Liberation Front is a group of activists who actually provide tactics for illegal defacement of billboards on their website. Though these groups have had many impacting actions, not all successful challenges are demonstrated through such radical means.

Many groups, such as Scenic America have taken stands against billboards, describing them as distractions for drivers and reasoning them as causes for too much clearing of trees

and intrusions on natural landscape. The organization provides local activists with resources for defending the scenic conservation ethnic. Some projects work to protect forest or hillsides from cell towers while others encourage efficient land use and protect community character from modern corporate architecture.

Conclusion

The use of vernacular architecture in public space continues to be prevalent in our culture as the billboard medium proves its effectiveness and permanency in the American landscape. Billboards are rhetorical when you consider their potential in the act of influencing others. Through this perspective, billboards are examples of persuasion that examine social truths addressed to others, justified by reasons that reflect cultural values.

Any stroll down a Main Street or cruise along the highway will present a vast range of vernacular objects that contribute to our modern environment. Outdoor advertising—a medium that is available in more markets and exposed to more people than any other major advertising media—comprises only about one percent of all commercial signs, displays, and devices visible to the public eye. It is no wonder that immersion in such a dense landscape of symbols and structured messages such as billboards, that our society has recycled its values through this inescapable system of persuasive communication. Presented through imagery and designs, these values perpetuate themselves within a paradox; billboards are both ephemeral displays of public social values and reinforcements of the very values that the public is encouraged to live by.

Many factors have amplified this communicative medium to the extent that we experience it today. The automobile has transformed our society in so many ways; particularly the physical design of public space and ideals of mobility and independence that driving provides. The expansion of road systems during the thirties and fifties, certainly amplified road culture and the outdoor advertising industry's potential for market. Along with television and other technologies, the American literacy has quickened, demanding massive stimulation in efforts to escape boredom. This evolution has substituted domestic spaces and reinvented consciousness of communication. Billboard aesthetics have demonstrated this evolution of cultural values in the symbolism and designs they embrace, serving as artifacts of our past.

A keener understanding of the relationship between rhetorical perspective and the objects articulated and reified through discourse may help generate enlightenment of our consciousness in the present, and ultimately the future. In other words, one studies historical artifact not to re-create or re-view the past but in order to better understand the present and more directly impact the future. By learning to approach historical documents and popular cultural artifacts from this perspective, perhaps some consensus can reached on the most suitable outlines for future development. Similar to the ideology of New Urbanism, vernacular structure needs to be carefully considered, as it is obvious how much impact it has on social perception within a public space. Of course, the inevitable interests of corporate tradition and commercial profits will likely always complicate the purity of such intentions.

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Expression Methods and Preliminary Purification of HIV-1 fusion protein GST-vif

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The HIV-1 protein, vif, is essential for HIV-1 replication in T lymphocytes and macrophages.[1,2] Recently, it was discovered that retroviral hypermutation of first strand viral DNA by the human protein hA3G occurs upon infection by HIV-1 virions. hA3G is a virion-encapsidated host protein able to deaminate minus-strand reverse transcript deoxycytosine bases to deoxyuracil.[3,4] These C to U mutants are copied as G to A mutations by reverse transcription of the coding or + strand, thereby introducing catastrophic mutations into proviral DNA that blocks its infectivity. However, the interaction between vif and hA3G results in a dramatic reduction in the amount of hA3G encapsidated into virions. In fact, hA3G association with vif has been well documented, and the ensuing lack of hA3G incorporation into HIV-1 progeny virions has been ascribed to vif-mediated polyubiquitination of hA3G, leading to its rapid degradation.[5,6] Specifically, vif targets hA3G through formation of an SCF-like E3 ubiquitin ligase complex that includes Cullin5, Elongin C and Elongin B. The structure of the vif-BC-Cul5 complex has been demonstrated to form a novel SOCS-box-like motif, which acts as an E3 ligase for hA3G in vitro.[8,9] The new SOCS-box motif (Figure 1) varies from the originally identified SOCS-box[7] by a cysteine residue replacement of alanine in the consensus sequence, as well as two identified cysteine residues outside the SOCS-box that are required to interact specifically with Cul5, suggesting that other protein interfaces between vif and the SOCS-box Elongin C interaction exist.[10]

Furthermore, recent studies have shown a complementary mechanism for vif-mediated inactivation of hA3G, involving direct vif-hA3G binding. D128K substitution in hA3G impaired protein-protein interaction between the C-terminal of vif and hA3G.[11] In addition, the vif-BC-Cul5 complex interaction with wild type hA3G was inhibited by the D128K mutant, which does not interact with vif at all. Other vif mutants found to be defective were SLQ144/146AAA, which is a mutation in the vif-SOCS-box motif and C114S, C133S vif mutants that inhibit E3 ligase activity of the Vif-BC-Cul5 complex.[8] In experiments involving BC-box vif mutants, it has been shown that vif fails to recruit Cul5-E3 ligase; thus hA3G is not suppressed, yet vif-hA3G interactions are maintained.[9] These results suggest that E3 ubiquitin ligase activity of the vif-BC-Cul5 complex is necessary for the inhibition but not for the binding of hA3G by vif.[8]

The molecular characterization of HIV-1 proteins as well as those of their host partners such as hA3G is fundamental to elucidating the viral mechanism of action. In order to design rational therapeutic solutions that target the HIV-1 virus, the complete three-dimensional structures of viral and host proteins must be understood at the molecular level. This information is carefully collected through a multi-step process involving protein expression, protein purification, and ultimately, X-ray diffraction methods. With crystallization data, the active site structure(s) of a protein can be modeled in detail and can provide insight into the effects of mutants on the emergence of drug resistance. For example, the structure of HIV-1 protein protease double mutant dimer C95M/C1095F was determined

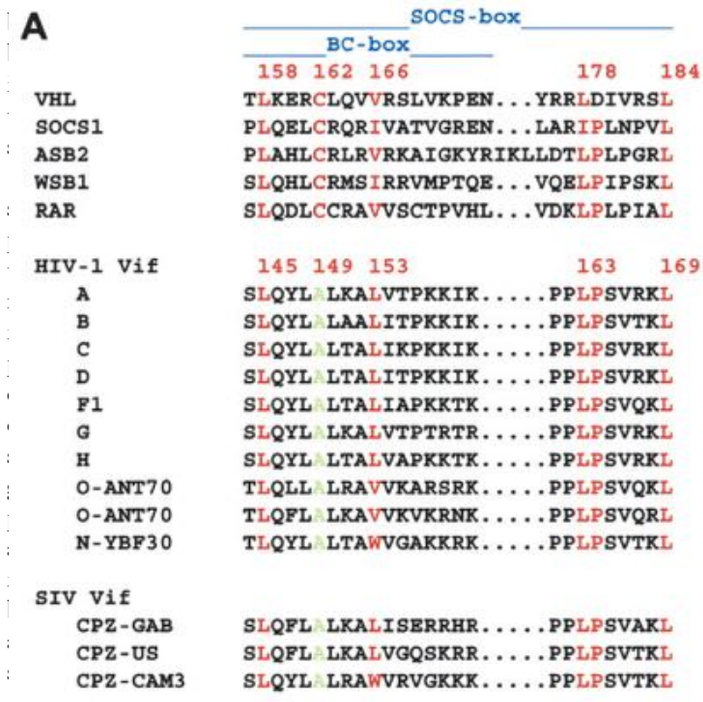

Figure 1. Vif of HIV-1 and closely related SIV cpz viruses contain a SOCS-box-like motif (10).

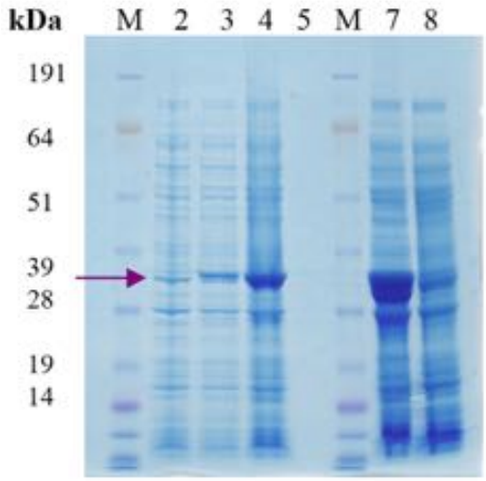

Figure 3. Original GST-vif expression profile. Lanes 2-4 illustrate uninduced, 1h and 2h after induction cell samples respectively. Lane 7 is the insoluble phase after lysis and Lane 8 is the soluble phase after lysis. Sequestering of GST-vif into insoluble inclusion bodies is seen in Lane 7 when compared to the supernatant in Lane 8. The majority of GST-vif remains in the pellet. Lanes marked M are mass markers. The arrow indicates GST-vif.

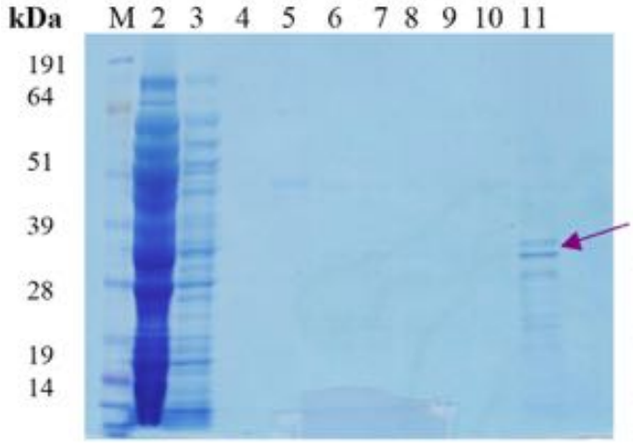

Figure 4. GST column elution using vif-GST from original expression. Lane 2 is column flow through and Lanes 3-5 are washes. Lanes 6-10 are successive elutions and Lane 11 is resin after elution. Minimal amounts of GST-vif are seen to bind to the resin and a scant amount is eluted. The arrow indicates GST-vif.

transformed with GST-vif cloned by PCR into a commercially obtained pGEX (lactose inducible) vector from Amersham Biosciences. Kanamycin (Kan) and Chloramphenicol (Cam) resistance genes were used for antibiotic selection. Plates were streaked overnight using Cam and Kan-containing LB agar. Plates were then stored at 4°C until needed. The first inoculation was performed using 5 ml LB, 12 µl Kan (30 mg/ml) and 5 µl Cam (34 mg/ml). These were placed in a shaker at 37°C and 225 rpm overnight. The culture was expanded to 500 ml LB, and final Kan and Cam concentrations of 72 µM and 34 µM, respectively. The 5 ml culture was spun down at 12,000xg, resuspended in 0.5 ml of fresh LB, and then added directly to the 500 ml LB. The flask was placed into the shaker at the original setting until it reached OD_{600} nm of 0.6. The culture was then induced with 1 mM IPTG (artificial lactose-mimic substrate) and grown at 30°C for 2 hours. Subsequently, the cells were collected by centrifugation at 1,789xg for 10 min and the pellet was frozen in liquid nitrogen and stored at -90°C.

A few modifications were made to the above procedure: 1) LB was modified to contain 1 mM betaine and 0.5 M NaCl; 2) Induction was done at OD_{600} nm of 0.8 with 0.5 mM IPTG; and 3) the culture was heated to 47°C immediately following induction for 20 min and then grown overnight at 20°C to slow protein expression process.

Expression was done according to the modified method but without heat shocking the cells after induction.

Bacterial Cell Lysis

Frozen cell pellets were weighed and incubated in 4 ml Lysis Buffer/1 g of cells (Lysis Buffer contained: 0.5% Triton-X-100, 50 mM Tris pH 8.0, 10 mM BME, 1 mg/ml lysozyme, 5 µg/ml aprotinin, leupeptin, pepstatin A, 2 mM benzamidine, and 1 mM PMSF) that were then diluted 1:10 with 1xPBS and left for 30 min on ice. The cells were then sonicated and incubated

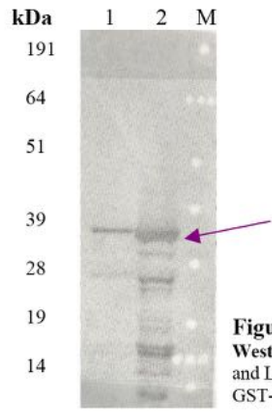

Figure 5. Original GST-vif expression Vif Western Blot. Lane 1 is concentrated GST-vif, and Lane 2 is the flow through off the GST column. GST-vif is present in both samples.

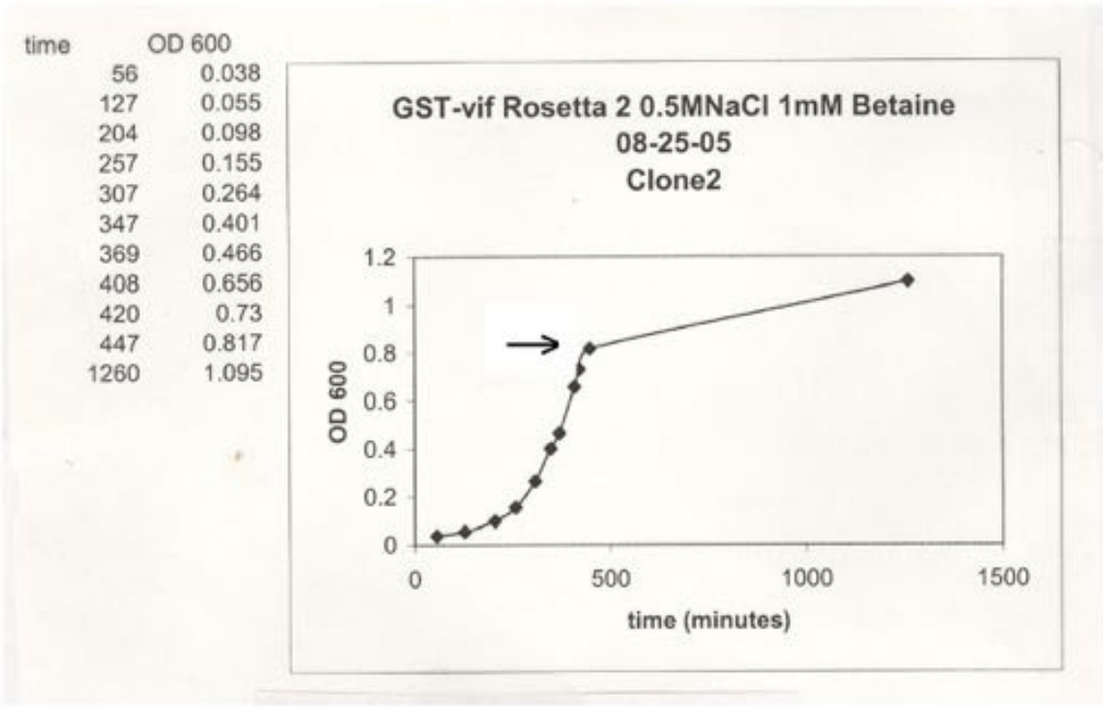

Figure 6. Sample GST-vif Growth curve. Representative of E.coli growth with pGEX vector. The arrow represents the time point of induction with IPTG as well as heat shock.

in 1 ml Nucleotide Digestion buffer/1g of cells (Nucleotide Digestion Buffer contained: 0.5% Triton-X-100, 2 mM ATP, 10 mM $MgSO_4$, 33 µg/ml DNase 1, and 33 µg/ml RNase A) for 10 min at room temperature. Finally, the lysate was clarified at 8,000xg for 15 min and the supernatant was pooled. After lysis, samples were collected and run on an SDS-PAGE gel using an XCell SureLock system (InVitrogen).

Expression Solubility Profile and Vif Western Blotting

Time points during expression were taken immediately after heat shock at 10 min, 20 min, 50 min, 80 min. 110 min, 140 min, 170 min, 210 min, 8h, and 21h. Cell pellets were then analyzed by SDS-PAGE using an uninduced sample as a control. Another gel was run, transferred in an XCell II Blot Module (InVitrogen) onto a PVDF membrane using the manufacturer's protocol, and a western blot was preformed. A monoclonal primary antibody (#6459 from the AIDS Reagents Repository) reactive with vif was used in a 1:1000 dilution into 1% powdered milk, followed by a 1:2500 dilution of goat anti-mouse secondary antibody containing 1% BSA. The membranes were then scanned using Enzyme Chemifluorescence on a Storm 860 imaging system (GE Healthcare). Peaks were then analyzed by Image Quant software.

Purification by Glutathione Sepharose 4B

Purification of the GST-vif fusion protein was conducted as described by the manufacturer using glutathione Sepharose 4B (GE Healthcare). A 5 ml column was filled with 1 ml of 75% Glutathione Sepharose 4B slurry. The beads were then washed with 25 ml 1xPBS pH 7.3. Bacterial lysate was added and nutated for 1h at 4°C. Flowthrough was collected and the column was washed with 3x5 ml 1xPBS. After washing, 10 mM reduced glutathione at pH 8.0 was passed over the column as 5 bed volumes. The eluate was analyzed by SDS-PAGE.

GST-vif Concentration

After elution, an SDS-PAGE gel was run and a Bradford assay was performed to quantify the concentration of protein in the elutions. The most concentrated elutions were then pooled and dialyzed overnight in dialysis buffer. Pooled and dialyzed material was subjected to further concentration by use of a stirred cell (Amicon) pressurized with Argon gas using a 5,000 NMWL cutoff membrane. The sample was brought to a volume of 0.75 ml.

Modified Glutathione Sepharose 4B Elution

To elute the GST-vif fusion protein, 10 mM, 50 mM and 100 mM concentrations of reduced glutathione were used in 1xPBS buffer pH 8.0. Elutions were 0.75 ml (1 bed volume each) of 10 mM, 2x50 mM, and 2x100 mM reduced glutathione respectively and were allowed to nutate at 4°C for 10 min each. Aliquots of 5 µl were removed from each elution, as well as a resin sample for running on an SDS gel.

Results

Standard Expression of GST-vif

The initial expression of GST-vif resulted in high concentrations of GST-vif in the pelleted material prior to purification. As seen in Figure 3, the growth lanes 2-4 indicate that GST-vif is in fact being expressed (GST-vif runs just below the 39 kDa mark). However, after lysis, the majority of expressed protein is in the insoluble phase (Lane 7) rather than in the supernatant (Lane 8). Furthermore, GST-column elution, as seen in Figure 4, indicates that very little protein is binding to

the column. Vif western blot of stirred-cell concentrated GST-vif confirms that GST-vif has been expressed (Figure 5).

Modified Expression of GST-vif

Heat shock improved quantities of soluble GST-vif by inducing expression of specific chaperones involved in protein folding and stability. After heat shock, betaine and NaCl addition acted to ionically stabilize the different charges on the protein's surface upon expression and during folding. Figure 6 demonstrates good bacterial growth under the modified conditions. Furthermore, to prevent protein aggregation, IPTG concentration was reduced from the standard 1 mM to 0.5 mM along with growth temperature and expression time, slowing down GST-vif expression. These methods produced more soluble GST-vif, as seen in Figure 7, represented by lanes 1 and 2 (the other lanes will be alluded to upon discussion of purification), illustrating respectively the insoluble and soluble phases after lysis.

Expression of GST-vif without heat shock immediately following induction results in GST-vif partitioning in the insoluble phase. In Figure 8, Lanes 1 and 2 show GST-vif expression in uninduced and induced samples. Lanes 4 and 5 are the insoluble and soluble phases following lysis, respectively. Furthermore, the sample was loaded onto a GST column and GST-vif elutions were undetectable by Commassie stain. Thus, without heat shock, GST-vif does not fold into its soluble, native structure. This observation supports the hypothesis that specific chaperones from the Hsp family of proteins aid GST-vif in its three-dimensional folding process. The Hsp chaperones are only expressed at certain high temperatures and stabilize newly expressed proteins by providing folding microenvironments and by binding hydrophobic domains.

Expression Solubility Profile

According to the modified method of GST-vif expression, time points were taken at induction, 10 min, 20 min, 50 min, 80 min. 110 min, 140 min, 170 min, 210 min, 8h, and 21h. Figure 9, Lanes 3-23 show increasing concentrations of soluble GST-vif over time. The insoluble phase is followed by the soluble phase for each time point beginning with Lane 3. Lane 2 is a sample of cells before induction. A western blot for vif expression was preformed on the samples from the kinetic analysis (Figure 10). Figure 10 better quantifies the amount of soluble protein at each time point, which is greatest after 8 hours of expression (Lane 20).

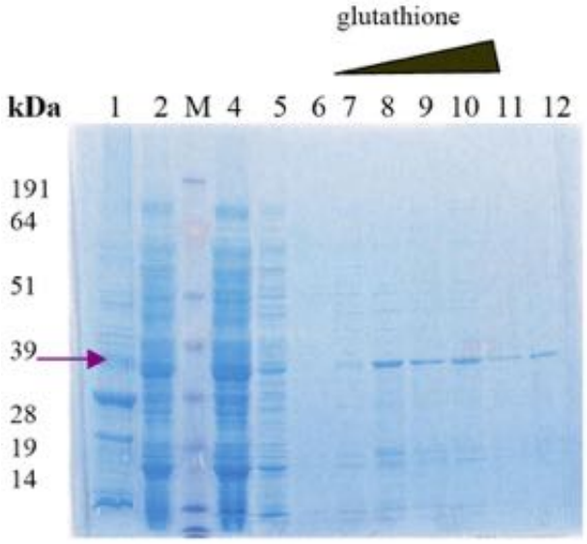

Figure 7. Modified GST column elution. Lane 1 is the insoluble phase after lysis. Lane 2 is the soluble phase after lysis. Lane 4 is flow through and Lanes 5-6 are washes. Lanes 8-11 are elutions of 10 mM, 50 mM, 50 mM, 100 mM and 100 mM glutathione respectively. The last lane is GST-vif remaining on the resin after modified elution. When compared to Figure 10, much more GST-vif is eluted off the resin with higher reduced glutathione concentrations. The arrow represents GST-vif.

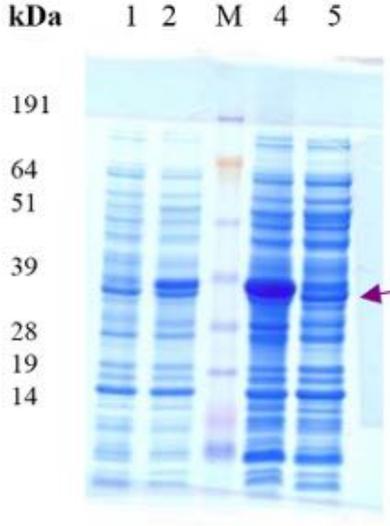

Figure 8. Modified expression protocol without heat shock. The first lane is a sample of cells before induction and the second is after induction. Lanes 4-5 show the insoluble and soluble phases after lysis respectively. GST-vif is most prominent in Lane 4, the insoluble phase. The arrow indicates GST-vif.

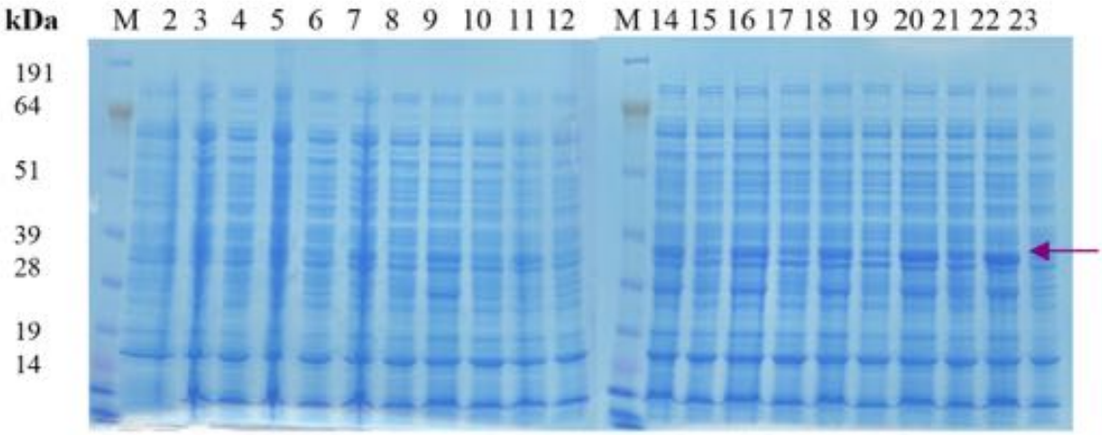

Figure 9. Modified expression solubility profile. Lane 2 is a sample of uninduced cells, followed by time points taken successively during one expression period. Lane 3 is an insoluble phase taken at 10 min followed by the soluble phase in Lane 4. This pattern repeats with time points taken at 20 min, 50 min, 80 min. 110 min, 140 min, 170 min, 210 min, 8h, and 21h. The lane with the greatest amount of soluble GST-vif is Lane 21, corresponding to an 8 hour expression period. The arrow indicates GST-vif

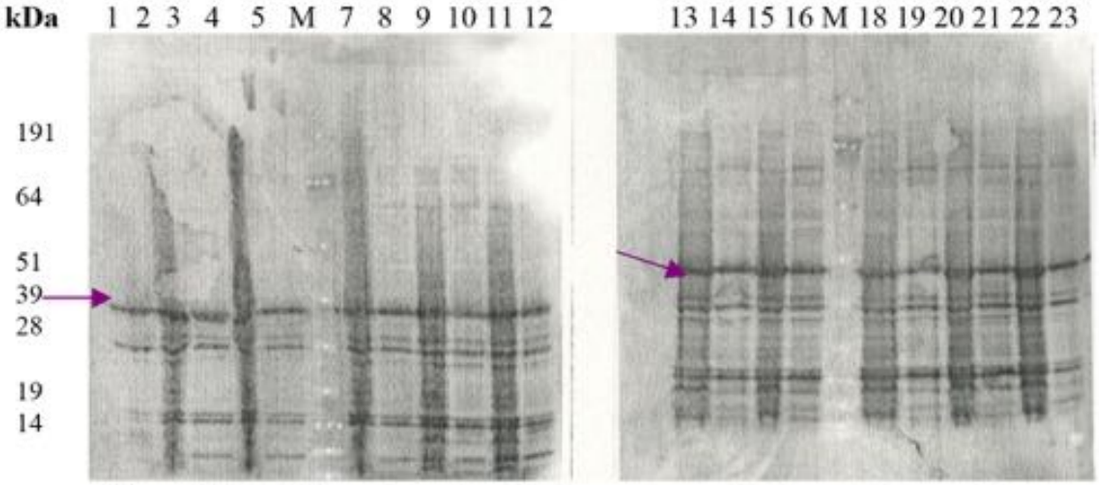

Figure 10. Vif Western Blot of modified expression profile (Figure 9). Lanes 1-23 correspond exactly to those in Figure 9, except for the placement of the molecular standards. This profile better quantifies the amount of soluble GST-vif, again illustrating the greatest amount in Lane 21, at the 8 hour expression time point. The arrow represents GST-vif.

Glutathione Sepharose 4B Column Elution

Following the manufacturer's procedure for Glutathione 4B elution (GE Healthcare), 10 mM reduced glutathione was used to compete with GST-vif. As seen in Figure 11, 5 elutions were resolved in Lanes 2-6. Lane 7 shows the amount of GST-vif left on the bead resin after elution. Though Lanes 2 and 3 show a larger amount of protein than the rest, the bead resin contains much more GST-vif than any other lane. Thus, the elution buffer is a poor competitor at 10 mM concentration to GST-vif. Consequently, higher concentrations of reduced glutathione were used to elute GST-vif. Figure 7 illustrates the use of 10 mM, 2x 50 mM and 2x 100 mM column elutions (Lanes 7-11). Lane 12 in Figure 7 reveals the GST-vif remaining on the bead resin. In comparison to the amount of protein left on the resin in Figure 11, higher concentrations of reduced glutathione in the elution buffer were more effective in eluting GST-vif from the column.

GST-vif Concentration

After concentration to 750 µl of GST-vif elutions in a stirred cell from lanes 3-5 in Figure 11 (Lane 3 of Figure 12), a vif western blot was run to confirm the presence of vif (Figure 13). Bradford Assays performed on the pooled elutions before and after concentration show more than a threefold increase in concentration (Figures 15 and 16).

Discussion and Conclusions

Further understanding of the HIV-1 vif protein requires successful expression methods that yield sufficient amounts of GST-vif to be obtained. The modified expression procedure employed in this paper addresses the issue of insoluble protein in three ways. First, it has been suggested that higher solute concentrations help stabilize native proteins in solution by interacting with their charged surfaces.[16] Secondly, heat shock

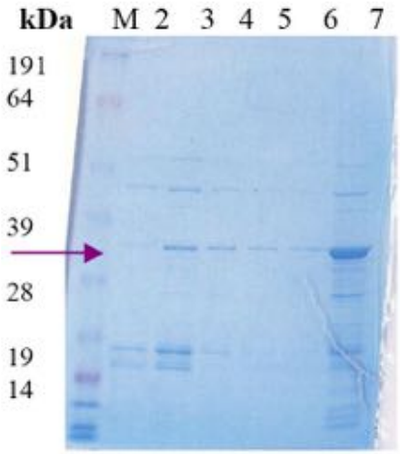

Figure 11. Manufacturer's protocol for 10 mM reduced glutathione GST column elution. As described by GE Healthcare, 5 bed-volume elutions of 10 mM reduced glutathione were passed over GST-vif as seen in lanes 2-6. Lane 7 contains the resin after elution. Large amounts of GST-vif remain on the resin after elution. Only minimal amounts are eluted. The arrow indicates GST-vif.

stimulates the expression of proteins known as chaperones, such as DnaK, DnaJ, GrpE, GroEL and GroES.[18] Chaperones and other heat shock proteins aid protein folding in the following ways: by providing specialized folding microenvironments, by binding to hydrophobic domains of unfolded proteins in order to stabilize them and by aiding in protein transport. Third, extension of the expression time by lowering the growth temperature reduces protein incorporation into insoluble inclusion bodies by reducing the rate of protein expression and thus lowering the probability of aggregate formation between nascent proteins.[15,16] The addition of 1 mM betaine and 0.5 M sodium chloride alone did not improve protein solubility (Figure 8), but in combination with heat shock and expression at lowered temperatures over a longer period of time, these additives increased expression (Figure 7). In the future, this method will be adapted and recommended to others with similar expression issues.

Following successful expression of GST-vif, purification ensues. Competitive binding with respect to affinity chromatography is often unpredictable when dealing with proteins whose native structures are not known. Many possibilities involving the folding of GST-vif may explain its unusually high affinity for the GST column (see Figure 11 Lane 7). Because of the varying nature of protein folding, standard procedures such as those given in the GE Healthcare GST manual are unlikely to be representative of all possible native protein structures and their interactions with glutathione sepharose 4B. The problems encountered in eluting protein from the GST column suggest that the concentration of reduced glutathione is not sufficient to successfully compete with the GST-vif bound to the column. Through inference, elution buffer concentrations of reduced glutathione were increased and resulted in higher concentrations of eluted GST-vif. A comparison of Figures 7 and 11 illustrates that higher amounts of reduced glutathione have a greater ability to elute GST-vif. Future methods will include preliminary elutions of reduced concentrations of reduced glutathione (i.e. 10 mM and 30 mM) in order to exploit purification steps by washing off proteins unbound to the column. Examination of Figure 7 shows that both high and low molecular weight contaminants exist on the column after washing. The first 10 mM reduced glutathione elution actively rids the beads from contaminants with a minimal loss of GST-vif. Thus, a few elutions of low reduced glutathione concentrations will be helpful steps to purify GST-vif, and will be followed by higher concentrations to elute GST-vif itself.

This study focused on the vif protein from HIV-1 since it appears to defeat the innate anti-viral protein from hA3G. Rescuing hA3G represents an exciting new therapeutic strategy since all HIV-1 therapeutics currently being used only target traditional viral proteins. In 2004, the WHO estimated about 40 million people worldwide were infected with HIV-1 and about 3.1 million with HIV-1 died. At present, multi-drug therapies have decreased the HIV-1 death rate by reducing viral expression in the host. However, these therapies cost about $15,000 per person per year, making them primarily accessible to medically insured patients in developed countries. It is estimated that the US economy alone spends about $3-6 billion dollars a year to control the virus, and about $514 billion is spent globally. Until an inexpensive, effective therapeutic is developed, the HIV-1 virus will relentlessly spread worldwide. It is believed that before long, many countries in Africa, Asia, and Eastern Europe, which cannot afford the current medications, may become completely devastated before a solution is found.[13] Significantly, in the year 2005, 16-40% of new HIV-1 infections will be resistant to one or more existing forms of viral therapy.[19] Hence it is of paramount importance that a new class of therapeutics be developed.

Results from the expression and binding experiments enabled an understanding that GST-vif folding needs to be aided by chaperones to prevent it from being packed into insoluble inclusion bodies. Furthermore, the high affinity of GST-vif for the GST column also provides structural folding information. The native state of GST-vif must interact favorably with glutathione sepharose that has two negatively charged groups and one positively charged group on its surface. Future steps include proceeding with expression and purification of GST-vif with the goal of purifying vif. This will consist of PreScission Protease cleavage and further purification steps. Further purification will be obtained by running the sample through an S-100 size exclusion column and/or HPLC. PreScission Protease cleavage will involve off-column cleavage held at varying temperatures and solution conditions to maximize cleavage results. After further purification, crystallization using hanging drop vapor diffusion, as well as the batch under oil method efforts will initiate along with binding assays involving hA3G, and Elongin B and C using the GST-tag or other affinity devices.

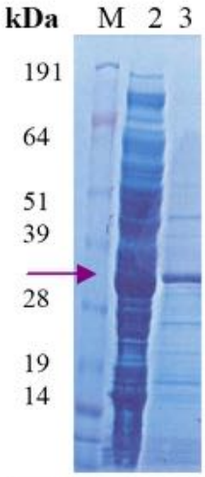

Figure 12. GST-vif stirred cell concentration. Lane 2 corresponds to flow through from purification and Lane 3 is concentrated GST-vif using Lanes 3 and 4 from figure 11.

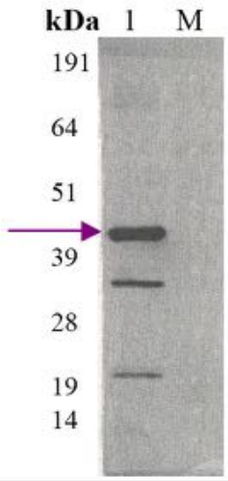

Figure 13. Vif Western Blot of concentrated GST-vif (Figure 12). GST-vif was taken after concentration (Lane 3, Figure 12) and blotted for vif, which is clearly illustrated.

µg/ml	OD595	200ul Bradford Reagent
0	0.000	Blank (zero by default)
5	0.159	<-- Value from standard
10	0.454	<-- Value from standard
15	0.599	<-- Value from standard
20	0.764	<-- Value from standard

µl sample/ml	OD595	
5	0.085	<-- Small µl OD reading
15	0.145	<-- Large µl OD reading

Best Fit Trendline (y = mx + b)	
m:	0.03936
b:	0.0016
R^2	0.9856

Replicates	x=(y-b)/m	Dilution	(The answer)
Small µl calc:	2.119	423.78	Average (mg/ml):
Large µl calc:	3.643	242.89	0.33

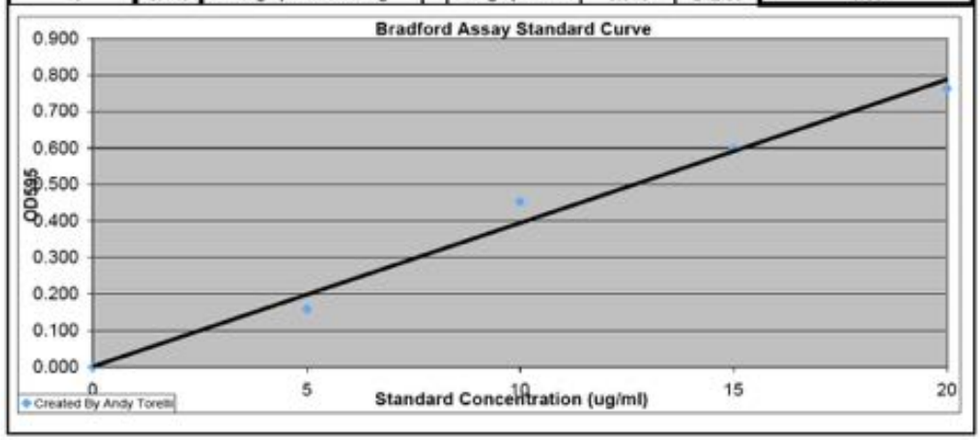

Figure 14. Bradford Assay on GST-vif before concentration. This line graph results from standard expression methods. Samples of 5 and 10 ul were used and OD was measured at 595 nm. The average concentration was 0.33 mg/ml.

Acknowledgements

Vif monoclonal antibodies were obtained from the AIDS Research and Reagent Program. Expression and concentration protocols were adapted with assistance from Celeste MacElrevey. I would also like to thank Dr. Harold Smith for his guidance, as well as Dr. Joseph Wedekind for providing me with the opportunity to study vif as a member of his research lab at the University of Rochester.

Abbreviations

Vif, HIV-1 viral infectivity factor; hA3G, human Apobec-3G, also known as CEM15; SCF, Skp1p-cullin-F-box protein complex, a ubiquitin-ligase; Cul5, Cullin 5; GST, Glutathione S-Transferase; Kan, Kanamycin; Cam, Chloramphenicol; LB, Luria Broth; IPTG, isopropyl-beta-D-thiogalactopyranoside; Tris, Tris Hydroxymethylaminomethane buffer; BME, beta-mercaptoethanol; PMSF, Phenylmethylsulphonylfluoride; PBS, Phosphate-buffered saline; PVDF, Polyvinylidene difluoride; BSA, Bovine serum albumin; HPLC, High Performance Liquid Chromatography; SDS PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; WHO. World Health Organization; rpm, revolutions per minute; g, gravity; NMLW, Nominal Molecular Wight Limit; Hsp. Heat shock protein; S-100, Sephacryl 100.

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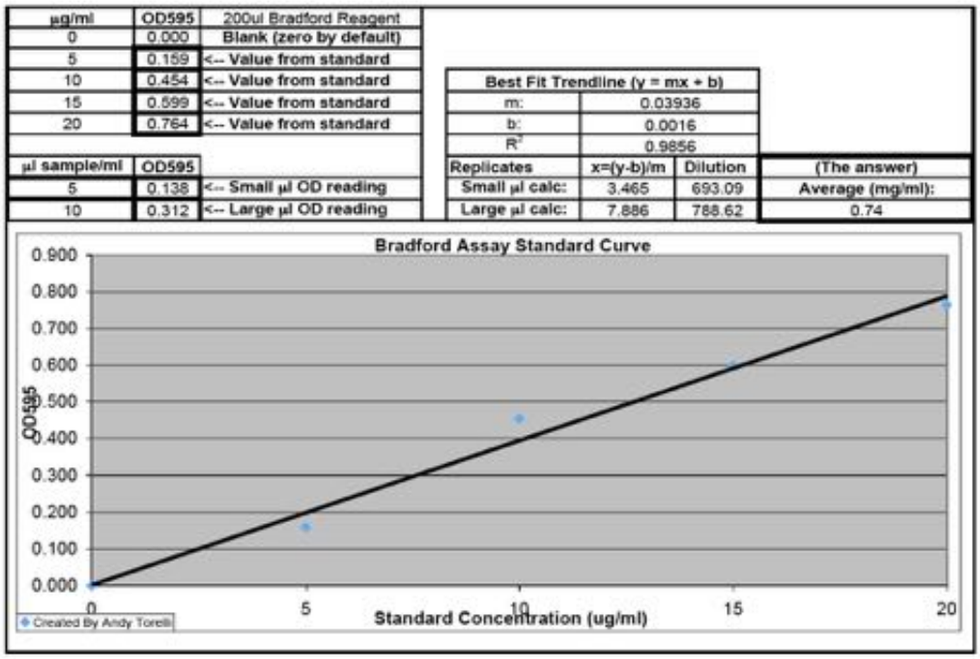

Figure 15. Bradford Assay on GST-vif after stirred cell concentration. The concentration increased from 0.33 mg/ml to 0.74 mg/ml with a corresponding 3-fold decrease in volume. As before in Figure 14, OD was measured at 595 nm, using 5 and 10 ul samples.

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Development of the Nodes of Ranvier in Wild Type and Dysmyelinated Mice

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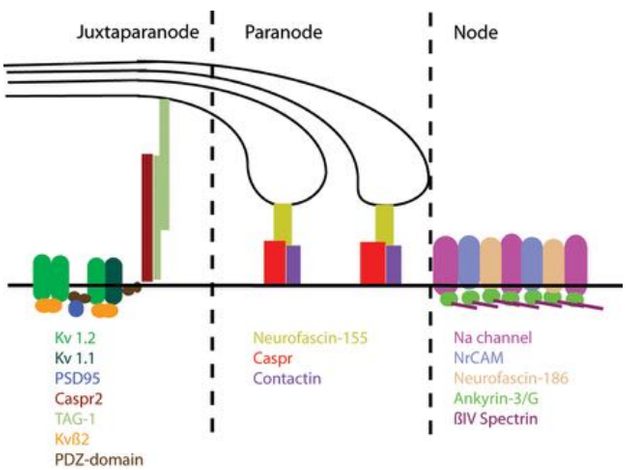
Myelin plays an important role in the saltatory conduction of action potentials in the nervous system. Myelin is composed of primarily lipids (~70%) and proteins (~30%) making it a good insulator for electrical current and the propagation of action potentials (APs) through the axons. The AP is propagated by saltatory conduction created by the influx/efflux of sodium and potassium ions. As the AP propagates along an axon, the strength of current decreases, but if the axon is myelinated the current can travel for a greater distance. In mammals, axons are ensheathed by myelin produced by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system. Not all axons are myelinated; their myelination depends on their diameter and projection trajectory. In the CNS, myelinated fibers are primarily found in the white matter tracts of the corpus callosum, cerebellum and cranial nerves.

At regular intervals, there is space between the wrappings of oligodendroglial processes, exposing the axon in highly specialized regions known as the nodes of Ranvier. The nodes of Ranvier are divided into three regions: node (~1μm), paranode, juxtaparanode (~5-15μm).[1] In order for AP propagation to occur properly, high densities of voltage-gated sodium (Nav) channels are found in the nodes and high densities of voltage-gated potassium (Kv) channels are found in the juxtaparanodes.[2] The paranode does not contain voltage channels, and acts as a diffusion barrier between the node and juxtaparanode. Aside from Nav and Kv channels, several other molecules have been identified at these sites that affect node of Ranvier formation (Figure 1). In the CNS of the mouse, myelination starts a few days after birth. During postnatal development, nodal molecules appear in an ordered manner following myelination.

Dysmyelination has a drastic effect on the development of nodes of Ranvier. For example, the *shiverer* and *jimpy* murine models have been used to look at how genetic dysmyelination affects formation of nodes of Ranvier. Most studies to date used teased fibers of the optic nerve as the primary source due to its accessibility, but some studies have looked at the corpus callosum and cerebellum as well. This review will focus on the development of the nodes of Ranvier in the CNS of mice, and how myelin deficiencies result in their deformation.

Figure 1: Diagram of protein interactions at the nodes of Ranvier.



Development of the node of Ranvier

Axoglial junction formation and clustering of sodium channels occur in the early stage of myelination.

The formation of axoglial junctions precedes the clustering of sodium channels, suggesting that the initial contact of oligodendrocytes with the axon is necessary for recruitment of nodal proteins. The axoglial junction is formed by the binding of the glial protein neurofascin-155 (nf-155) in a trans-formation to the axonal complex of Contactin-associated protein (Caspr) and Contactin.[3-6] At post-natal day 6 (P6) in mice, nf-155 is primarily found in the cell body and major branches of the oligodendrocyte, and by P8 it is diffusely distributed along the processes.3-6 The formation of the axoglial junction is regulated by the expression of nf-155. When nf-155 is knocked out, Caspr and Contactin no longer localize to the paranode.4 As nf-155 starts to gather at the paranode, Caspr is detected at the edges of processes on P7 labeled with myelin-associated glycoprotein (MAG), an early myelin marker. The appearance of Caspr precedes Nav channels by about two days and rapidly increases with a rate similar to that of Nav channels.[7] Contactin is found primarily in the paranode with some expression in the node, ~95% and ~5% respectively of total expression.[8] There are two isoforms of Contactin that differ in molecular weight.

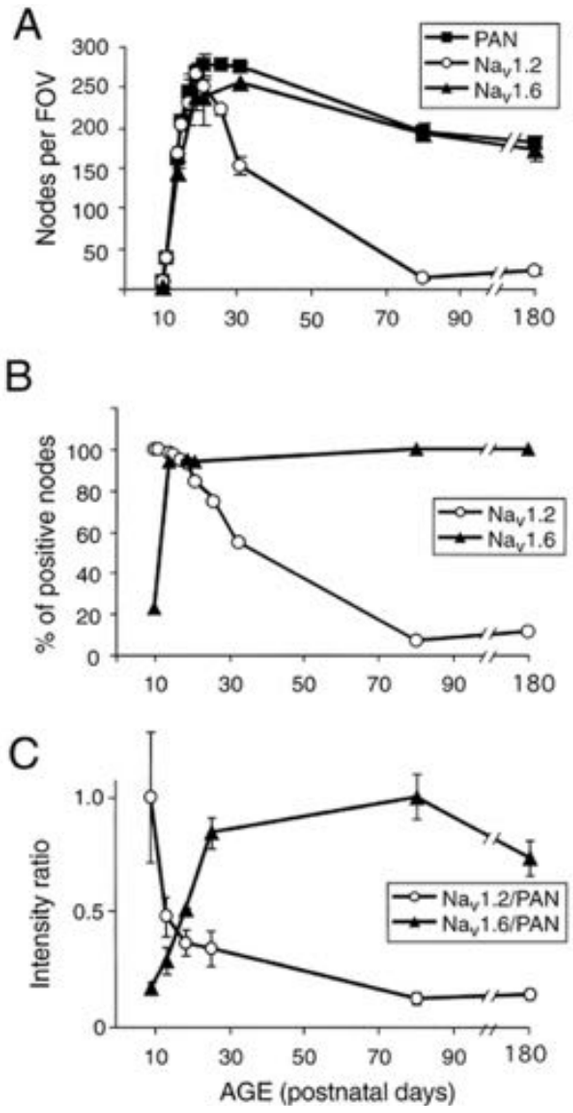


Figure 2: Quantification of the Replacement of Nav1.2 by Nav1.6 at Nodes of Ranvier in the Myelinating Optic Nerve. (A) The developmental time course of the number of PAN-stained, Nav1.2-positive, and Nav1.6-positive nodes per FOV [Field of View]. Images were taken from both proximal and distal portions of the optic nerve, and 3–5 FOVs were averaged for each developmental stage. Error bars represent ±1 SEM. (B) Proportion of PAN-labeled nodes that were positive for Nav1.2 and Nav1.6 at different ages. (C) Developmental change in the intensity of Nav1.2 and Nav1.6 immunofluorescence relative to the intensity of PAN immunostaining at the same node. Between 12 and 65 sites were measured for each age from Nav1.2/PAN and Nav1.6/PAN double-labeled images [15]

One isoform of Contactin is found in the paranode, and the other Contactin is found in the node.[9-11] Presumably Contactin in the paranode starts to appear at the same time as Caspr as the two molecules are bound together in a cis-formation.

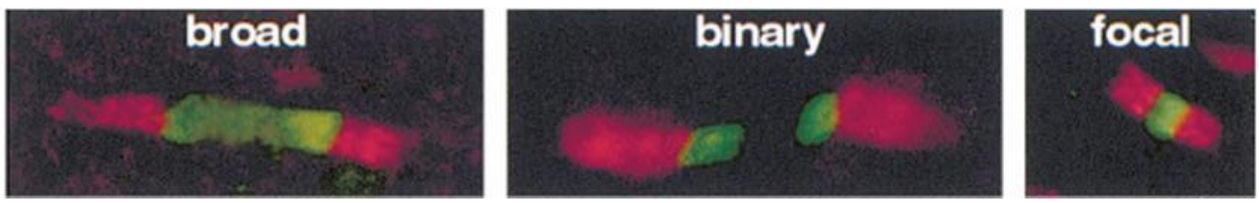
The recruitment of voltage-gated sodium channels to the nodes is preceded by other nodal proteins in addition to the formation of the axoglial junction. Neurofascin-186 is required for the localization of NrCAM, Ankyrin-3/G, and bIV-spectrin. When Neurofascin-186 is deleted, expression of these proteins remains the same, but does not localize to the node.[4] Neurofasin-186 and NrCAM have intracellular binding domains for ankyrin-3/G, forming complexes that anchor Nav channels to the node.[12]

Ankyrin acts as a bridge between membrane proteins, neurofascin-186, Nav channel, NrCAM, and the cytoskeletal protein βIV-Spectrin.[2,13] Prior to myelination, ankyrin-B is detected diffusely along the entire length of the axon[14], and is replaced with ankyrin3/G after myelination.[2] After myelination, ankyrin B is restricted to unmyelinated zones.[14,15] Due to low incidence of neurofascin-186 and ankyrin-3/G detection early in development, it is difficult to determine which protein is present at the clusters first. Ankyrin-3/G is detected at P7, with about two-thirds[13] colocalizing with Nav clusters at P9.[7]

Through interactions in the axon with ankyrin-3/G, neurofascin-186, βIV-spectrin, and NrCAM, sodium channels are stabilized at the nodes. Voltage-gated sodium channels are first detectable in the mouse optic nerve at P9-10.[7] At P9, about two-thirds of ankyrin-3/G clusters colocalized with βIV-spectrin, and the rest did not.[13] The appearance of βIV-spectrin at the same time as Nav channels suggests that the ankyrin-G does not anchor the nodal proteins until the channels are bound. Between P12-P22, there is a rapid increase in the number of channels and clusters formed (Figure 2A).[7,15] Nav channels are always adjacent to MAG processes, but they do not colocalize with MAG.[7] During the rapid increase in Nav channel number, the nodes can be defined as broad, binary or focal clusters (Figure 3).[7,15] During the period of increase, the change from broad (immature) to focal (mature) clusters marks the maturation of the node of Ranvier.[7]

The developmentally increasing clustering of Nav channels coincides with the change in the ratio of Nav1.2 to Nav1.6 expression. As the amount of myelin increases, the level of Nav1.6 expression increases, since broad and binary nodes tend to be Nav1.2, and focal nodes are Nav1.6.[15] Nav1.2 is detected on P9-10 at all early nodes with low expression of Nav1.6. Nav1.6 clusters at completion of myelination and whereas Nav1.2 clusters prior to completion of myelination. At P30, the end of myelination of the optic nerve, ~50% of nodes are still positive for Nav1.2, and ~90% are positive for

Figure 3: Nav1.6 Sodium Channels Are Found Primarily in Mature Nodes of Ranvier in the Optic Nerve. The confocal images were taken from sections double stained with anti-Caspr (red) and anti-sodium channel (green). (A) Examples of different node types that are thought to represent a developmental sequence of node maturation: a broad node (left), a binary node (center), and a focal node (right). The images were taken at P10 (broad node) or P21. The focal node with flanking Caspr staining on both sides represents a mature node of Ranvier. [15]



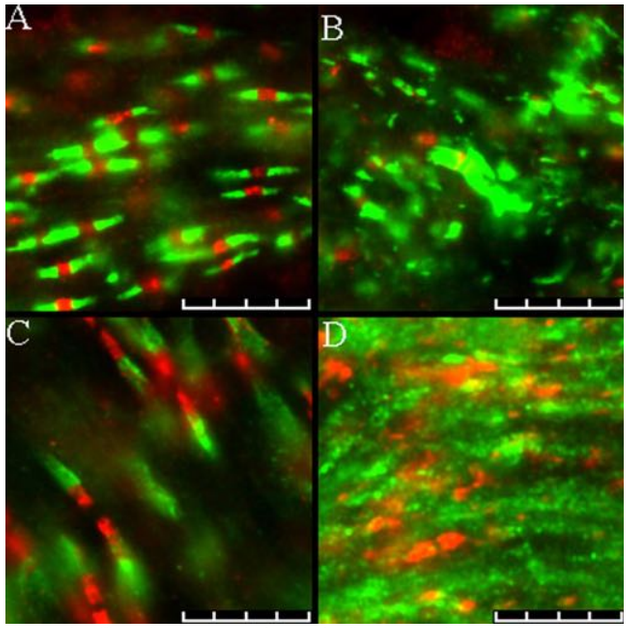


Figure 4: Effect of compact myelin on the formation of nodes of Ranvier. In the Corpus Callosum (A,B) and Cerebellum (C,D) of WT mice Caspr (green-A,B, red-C,D) Nav1.6 (red-A,B) and Caspr2 (green-C,D) have distinct borders as they form the nodes. Whereas the shiverer has very little Nav1.6 as a result of the aberrant Caspr localization. Caspr2 in the shiverer also has a diffuse distribution along the axons. Scale bars 10μm.

Nav1.6 (Figure 2B, 2C). By P80, the expression of Nav1.2 and Nav1.6 (Figure 4A) has reached the expression levels seen in adult mice.[15] Boiko and colleagues (2001) found that in the optic nerve the distribution of Nav channel distribution remained uniform in the unmyelinated portion, but focal in the myelinated portion, indicating that the presence and contact of myelin with the axon is necessary for nodal maturation.

Compaction of myelin and clustering of potassium channels occurs in the later stage of myelination.

Potassium voltage-gated channels are found beneath the myelin sheaths outside of the node in the juxtaparanode.[16,17] In the CNS, Kv channels start to cluster five days behind (P14) Nav channels and are only located in the juxtaparanode and not in the paranode or node.18 Kv channels mainly consist of Kv1.1, Kv1.2, and the Kvβ2.1 subunits.[19-21] Kv1.2 does not localize to the node by itself and is always found with Kv1.1, but Kv1.1 does not require Kv1.2 to be clustered at the juxtaparanode.[22] The presence of myelin also has an effect on the clustering of Kv channels. In the unmyelinated region of retinal ganglion cell axons, there is a uniform distribution of Kv channels throughout the axon.[15,23] In the myelinated region there is a discrete distribution to the juxtaparanode.[15,18,19] Compact myelin is needed for the localization of Kv channels. At P10, there is an increase in the number of axons that are myelinated, but the myelin is still loosely wrapped. Compaction of the myelin starts around P14 just as Kv channels start to localize. As the number of axons that have compact myelin increases, the number of Kv channel clusters increases.[23]

Prior to Kv channel clustering, Caspr2 is detected by P12 and may be involved in the initial recruitment of Kv channels. Caspr2 is a member of the neurexin superfamily that binds to TAG-1, stabilizing the juxtaparanode (Figure 4C).[24] TAG-1 is located on both the axonal and glial membranes.[25] The axonal and glial TAG-1 bind to each other in a trans-formation, while the axonal TAG-1 also binds to Caspr2.[26] Traka et al. (2003) and Poliak et al. (2003) showed that the localization of both proteins is dependent on the presence of the other. The Caspr2 knockout resulted in TAG-1 being absent from the juxtaparanode.27 Likewise, a TAG-1 knockout resulted in Caspr2 being redistributed to the internode.[26,27]

The presence of Caspr2 has a direct effect on the localization of Kv channels. Caspr coimmunoprecipitates with Kv1 channels24, but their interaction can be disrupted with most detergents.[28] Poliak et al. (2003) created a Caspr2 knockout mouse demonstrating its effect on Kv channels and development of the juxtaparanode. Kv channels in the absence of Caspr2 are redistributed to the internodal region but do not move toward the paranode. Kv1.2 expression remained similar to that of wild-type expression even though it was not localized directly to the juxtaparanode. Caspr2 is bound to Kv channels through a protein with a PDZ domain.[24,28]

PSD-95 has three PDZ binding domains that connect it to Kv 1 channels. It is not detectable at P14 before Kv channels cluster, but is detectable at some clusters by P17. At P17, there is some colocalization with Kv1.2 and Caspr2, which occurs at most sites by P22.[28] Rasband et al. (2002) created a mouse with a truncated PSD-95 mutation that does not affect the expression and localization of Caspr2 or Kv1 channels to the juxtaparanode. Although it appears to have a structural purpose, PSD-95 does not affect the maintenance of Kv1 clusters to the juxtaparanode.

Dysmyelinating diseases in the shiverer and jimpy mice result in abnormal formations of the node of Ranvier.

Compaction of myelin has a major effect on the formation of the node of Ranvier. The *shiverer* is deficient in MBP expression, resulting from a 5 exon deletion in the gene.[29] MBP is necessary for the compaction of myelin, forming major dense lines needed for proper axoglial interactions.[30-32] Neurofascin-155 is maintained at high levels in the *shiverer*, but tends to be found more in the cell body than the processes.[6] Caspr (Figure 4B, 4C) and neurofascin-155 colocalize at ectopic sites along the myelinated regions, but lack distinct boundaries.[6,7]

Due to the disruption in the axoglial junction in *shiverer*, the distribution of Nav and Kv channels are altered. The major difference in the Nav channel is the severe deficit of Nav1.6 channels. Nav channels in the *shiverer* are diffuse and irregular.[7] In the axons of retinal ganglion cells, the sodium channels make the switch from Nav1.2 to Nav1.6, but only in the initial segment.[15,33] Nav1.2 expression was increased in hypomyelinated regions, but distinction of broad nodes is difficult to make due to the uniform distribution along the axon.[15] Although the *shiverer* is lacking in amount of Nav1.6, it is still found at a few focal sites where Caspr is found to form a paranode (Figure 4B).[7] Expression of Kv1.1 and Kv1.2 are increased in cerebellar and subcortical white matter tracts.[34] Like the Nav channels, Kv channels have a uniform and aberrant distribution resulting in localization in the node and paranodal regions in addition to the juxtaparanode.[18,34] Caspr2 (Figure 4D) has a similar disruption in localization to that of the Kv channels. At sites where Caspr is localized, Kv channels cluster adjacent to each other,[2,18] suggesting the formation of

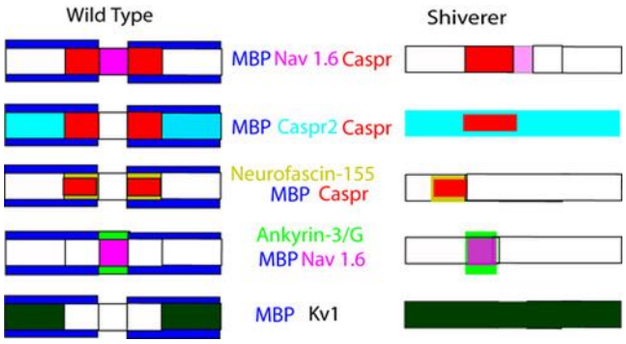


Figure 5: Distribution of antigens at the nodes in wild-type vs. shiverer mice.

axoglial junctions may be enough to induce clustering of Kv channels.

Jimpy mutant mice have a mutation in the *Plp* gene resulting in the death of many of the oligodendrocytes. Due to the lack of oligodendrocytes, the CNS is severely hypomyelinated. Kv channel expression in the *jimpy* is upregulated with a distribution resembling that of the *shiverer*.[23,34] Although a majority of the CNS has aberrant Kv channel distribution, the axons that do have compact myelin form correct channel clusters.[23] Not many studies have used *jimpy* mice as a dysmyelination model due to their short life span, which is about 25-30 days.

There are many factors that have been identified in the formation of the node of Ranvier, but there are also several unknown factors that may have an additional role. Oligodendrocyte contact with the axon may have an initiating role in node formation as seen in the models of *shiverer* and *jimpy*. Although most of the data on the development of the nodes of Ranvier in the CNS comes from teased fibers of the optic nerve it will be important to also look at the developmental time line in other white matter tracts.

There have been several remyelination studies in the PNS, but very few studies of remyelination in the CNS as a way to examine the reformation of the nodes after injury or disease. Our lab is currently using a remyelination model using both fetal and adult human oligodendrocyte progenitor cells (OPCs). The *shiverer* is used to test the ability of the OPCs to migrate and differentiate into mature oligodendrocytes in the white matter tracts of the corpus callosum35 and cerebellum (unpublished data). Both adult and fetal cells are successful in maturing to oligodendrocytes after migrating through the tracts35 as well as in forming functional axoglial junctions determined by colocalization of MBP and Caspr (unpublished data). We are further examining the ability of the human OPCs to fully reconstitute nodes of Ranvier.

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About the Author

Steven Schanz will receive his B.S. in Neurosience from the University of Rochester in May 2007. After graduation he plans to continue working in the Goldman lab.

jur: What is your research about / what applications does it have?

Our research is focused on using oligodendrocyte progenitor cells as a cell based therapy for treatment of congenital dysmyelinating diseases. We are using both fetal and adult cells from the ventricular zone, an area in the brain that continues to produce stem cells throughout adult life.

jur: How did you become interested in this area of research? / What motivated you to do this research?
I have had an interest in neuroscience for a long time before coming to UR. I did an internship at a research lab studying the effect of topography on the growth of neurons, but became more interested in the study of neural stem cells.

jur: How does this research relate to your major/future plans/interests?

As a neuroscience major the research allows me to see the correlation between what we learn in class and how it applies to research currently taking place. It also shows that the glia cells in the brain play a large roll in the functioning of the nervous system and it is not all about the neurons.

jur: While doing this research project, what was your biggest obstacle and how did you overcome it?

The biggest obstacle for our project is bridging the gap between mouse and human cells. As in any xenografts, transplants from one species to another, there is a certain level of host rejection. By immunosuppressing or using immunocompromised shiverer mice we have been able to get the human cells to survive longer and prolong the animal's life significantly.

jur: After completing your project, what do you think was your most fulfilling experience?

In April I will be attending the National Conference on Undergraduate Research to present.

jur: Any advice you can give to fellow undergraduates who would like to do this kind of research (or any other type of research)?

Get involved early on in undergraduate career. By starting early in sophomore year and working for the same lab through out your senior year you will be able to be more involved in the research. Starting early also makes it easier to do a senior thesis if one desires to.

The Effects of Adaptive Optics and a Scanning Laser Ophthalmoscope on RPE Cells

Katherine Shen, 2006

Advised by David R. Williams, Ph.D. and Jessica Wolfing

Center for Visual Science, University of Rochester

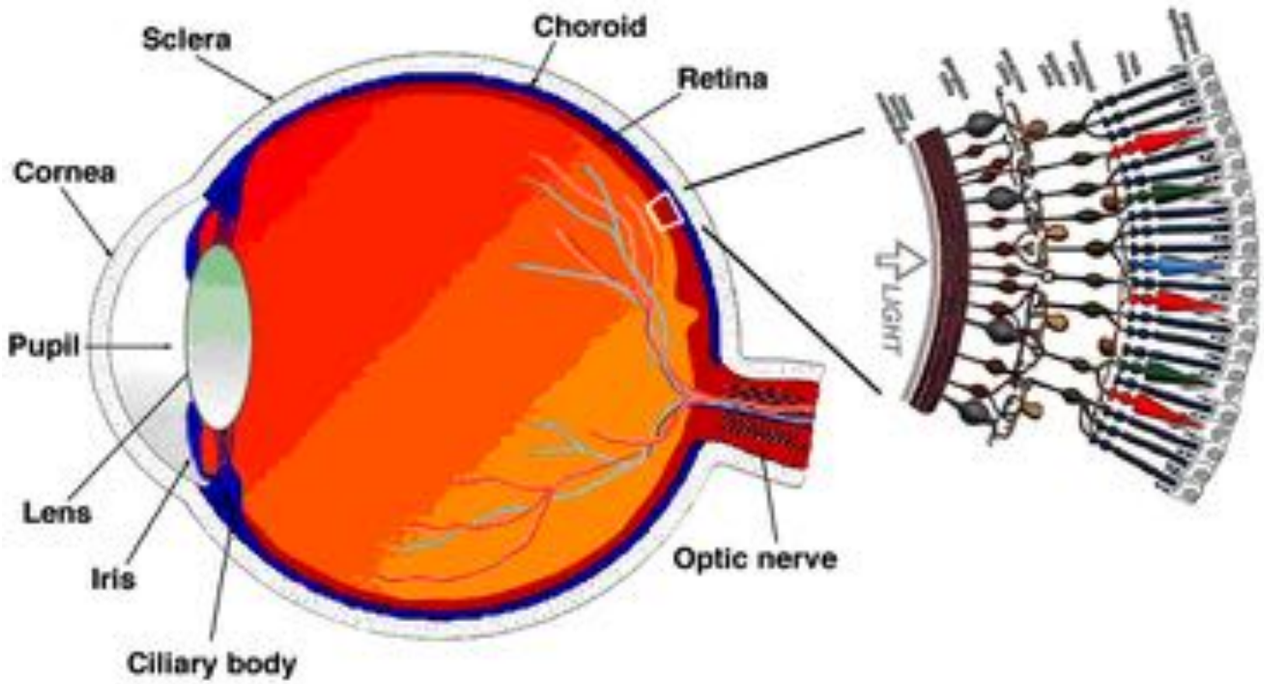
The human eye is complex and is composed of many cellular layers which play a role in the visual system. One of the layers in particular, is a single layer of cells called the retinal pigment epithelial (RPE) cells. They help support the function of the photoreceptor cells, providing nutrients and eliminating waste products. Numerous retinal diseases such as Stargardt macular dystrophy, Best's disease and cone-rod dystrophy[1] have been associated with the disruption of the structure and function of these cells. It is also found that these cells produce a variety of support mechanisms, which promotes the health and vitality of photoreceptor cells. Therefore, it is important to image and learn more about these RPE cells as they may lead to early detection and treatment of retinal diseases.

One of the ways to image these cells is through adaptive optics (AO) combined with fluorescence imaging. AO allows one to see small structures in the retina including individual photoreceptors, nerve fibers, capillaries, and blood cells.[2,3] Another technique using a scanning laser ophthalmoscope (SLO) provides the ability to take advantage of the intrinsic fluorescent properties of lipofuscin granulates which accumulate in the RPE as a natural byproduct of visual processes.[4,5,13-18] By combining the AO with the SLO, the RPE layer can be imaged in vivo at a high transverse resolution. The goal of this paper is to analyze the effects of this AOSLO method on the imaging of a primate, donor and a human retina.

Background

The light enters the front portion of the eye through the cornea and the lens. The light is focused onto the back of the retina which is composed of several layers of cells. These cells include the ganglion, amacrine, bipolar, horizontal, and photoreceptor, all of which are responsible for detecting light and sending the electrical signal to the brain for visual processing.

Figure 1: Schematic diagram of the eye.[10]



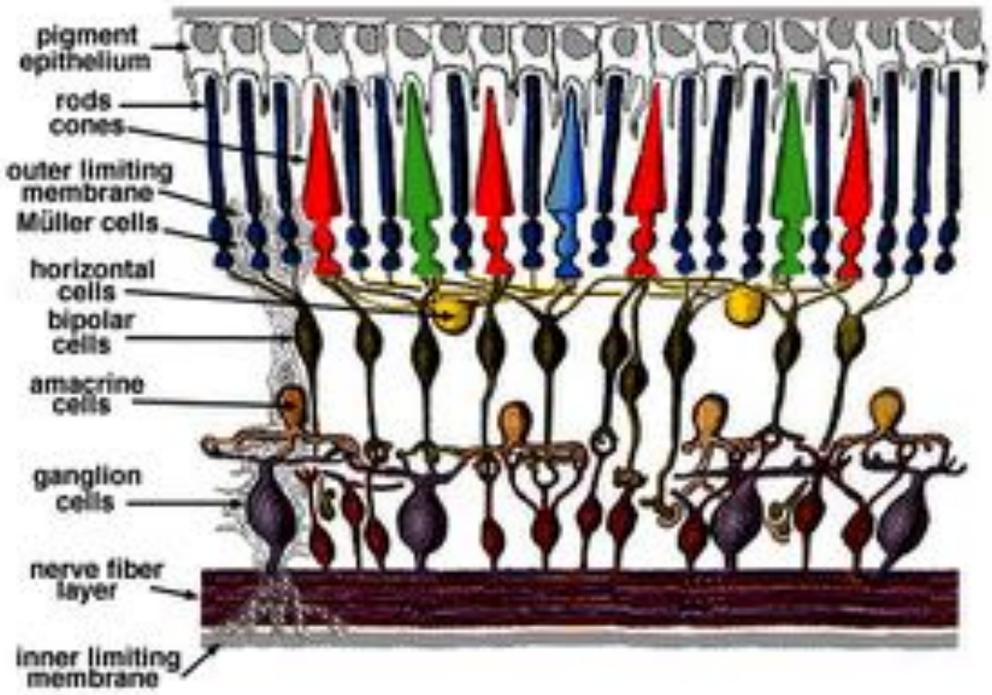


Figure 2: Simple diagram of the organization of the retina.[10]

RPE are responsible for supporting the photoreceptors. These layers are shown in Figures 1 and 2. The photoreceptors and the retinal pigment epithelium are the two deepest layers in the retina. The cells in front of the photoreceptors (ganglion, amacrine, bipolar and horizontal) are transparent allowing light that enters the eye to reach the photoreceptors.[6]

The photoreceptors are responsible for absorbing photons and relaying this signal to the inner cells of the retina. There are two types of photoreceptors: rods and cones, which play a role in night vision and color perception respectively. The cones are also responsible for high spatial resolution and are packed densely in the fovea (an highly specialized area), while the rods are located primarily in the periphery.

The RPE cells are responsible for absorbing scattered light in the retina which helps reduce any chance of scattered light being absorbed by the photoreceptors. These cells are located in the deepest layer of the retina, directly behind the photoreceptors, and form a monolayer throughout the entire retina. Because of the close connection with the photoreceptors, they are involved in supplying the photoreceptors with nutrients, participating in the regeneration of retina in the visual cycle and phagocytising photoreceptor outer segments.[7] They also form tight junctions between one another. The ones found in the periphery can be as large as 60μm in diameter while the ones in the fovea are between 10 and 15μm.[8]

Adaptive Optics Retinal Imaging System

The human eye has many imperfections, referred to as aberrations, which distort the light entering the eye. These imperfections in the eye can be measured by a wavefront sensor. In turn, most aberrations may be corrected by a deformable mirror (wavefront corrector). Figure 3 demonstrates the basic idea behind the adaptive optics. The wavefront sensor typically used in adaptive optics is called the Shack-Hartmann sensor, where a lenslet array samples the wavefront at a plane optically conjugate to the pupil of the eye, thus allowing measurement of the wave aberration of the optics of the eye.[1] The measurement and correction of the eye's aberration with Adaptive Optics has allowed high resolution retinal imaging of individual photoreceptors in vivo.

Adaptive optics technology was combined with the SLO by Roorda and colleagues in 2002 to provide high transverse resolution as well as axial sectioning capabilities.[1] Building on this technique, the David Williams' laboratory at the University of Rochester has built a system that combines the benefits of confocal, adaptive optics, multispectral, and fluorescence imaging.[9] The new fluorescence adaptive optics scanning laser ophthalmoscope (AOSLO) (Figure 4), consists of an adjustable scanning system, an adaptive optics system, two imaging lasers, and two detectors for simultaneous reflectance and fluorescence imaging. Fluorescence occurs when a molecule absorbs a photon at one wavelength, gets excited to a higher energy state, and then relaxes back to the ground state by emitting a photon of a longer wavelength than the one absorbed.[1] The light with the longer wavelength is the emitted fluorescence. Reflectance is the amount of light reflected from the image. With this method the smallest retinal capillaries and features of RPE cells have been seen.

Imaging individual RPE cells in vitro

In order to find and begin to understand the features of the RPE cells, a human donor retina was imaged in vitro under a conventional confocal microscope. The donor human retina was flatmounted on a microscope slide to allow en face1 imaging of the RPE. Fluorescence and reflectance images of an 81-year-old female donor's left retina were taken with a conventional confocal microscope shown in Figure 5.

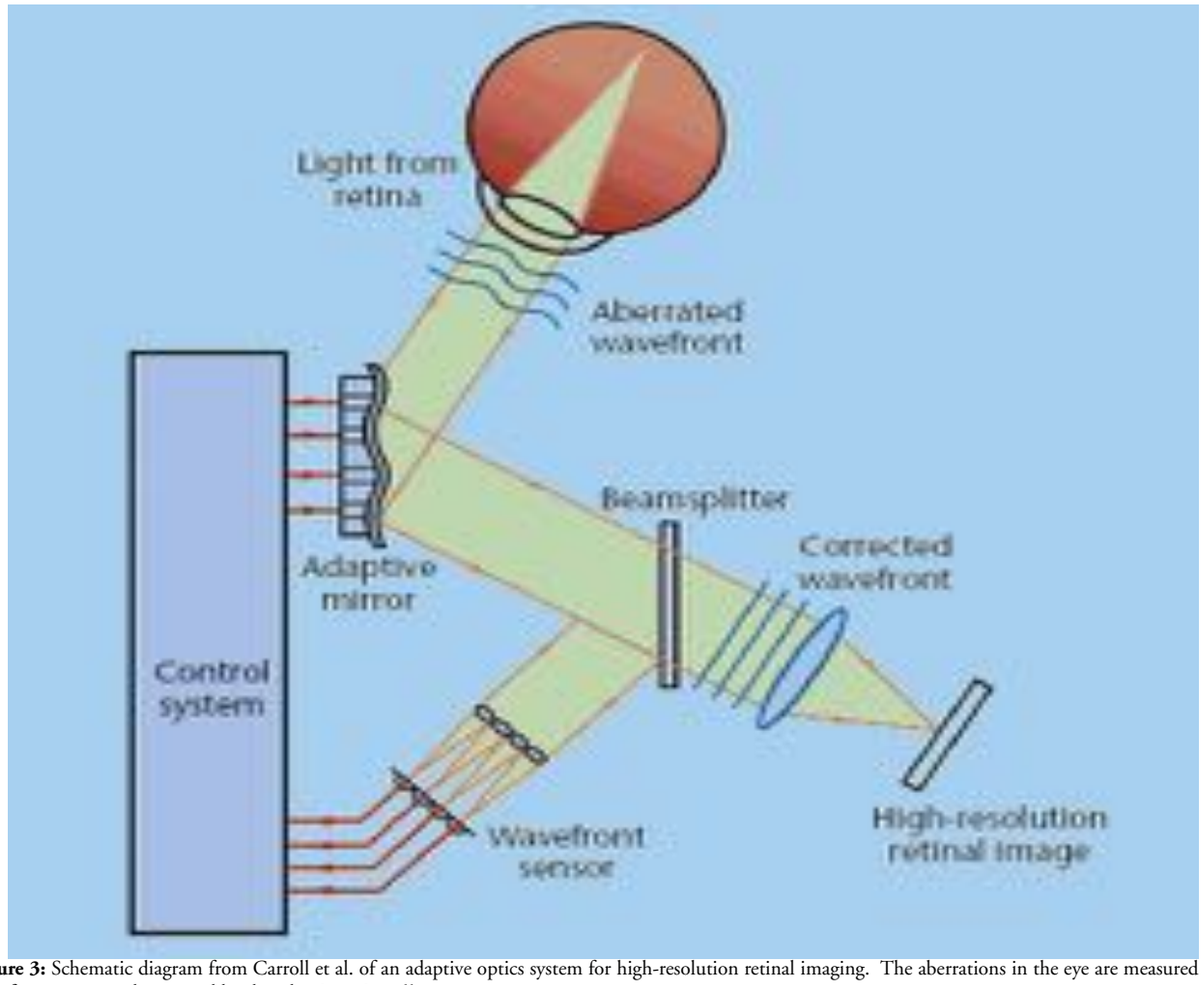


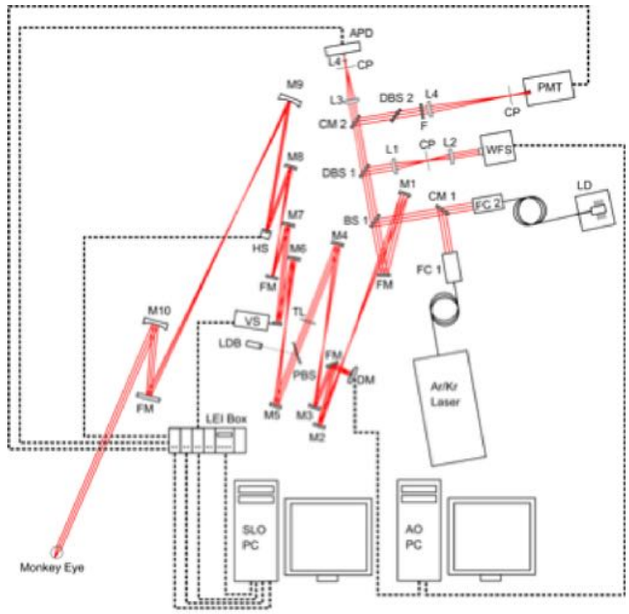
Figure 3: Schematic diagram from Carroll et al. of an adaptive optics system for high-resolution retinal imaging. The aberrations in the eye are measured by a wavefront sensor and corrected by the adaptive mirror.[11]

By looking at the fluorescence images, certain characteristics of the RPE cells were observed. The RPE cells appear as dark circles in the fluorescence image on the left of Figure 5 while in the reflectance image on the right, they appear white and saturated. This phenomenon is an indication that RPE cells contain autofluorescent properties of lipofuscin granules. Lipofuscin is composed of numerous autofluorescent molecules and it accumulates as a byproduct of phagocytosis of photoreceptor outer segments.[12] As one ages, lipofuscin is a major constituent of the RPE cells' cytoplasm, while the nucleus of the cells do not fluoresce.

Characteristics of the RPE mosaic from the donor retina

Images of the donor human retina were also taken using the AOSLO under both reflectance and fluorescence conditions. After collecting the images, the images were dewarped and summed. All of the fluorescence sums were then applied to a montage software program developed by a post-doctoral fellow in the Williams' lab where the program automatically aligns and superimposes two or more sums. As a result, a series of images are connected together to form a horizontal mosaic. This montage is shown in Figure 6. Similar characteristics of the RPE cells found in the conventional microscope were seen here. This shows that the AOSLO is capable of resolving the RPE cells and of detecting the low light levels obtained with autofluorescence.

Figure 4: Schematic design of the fluorescence adaptive optics scanning laser ophthalmoscope (AOSLO) system at the University of Rochester for high-resolution retinal imaging.[9]



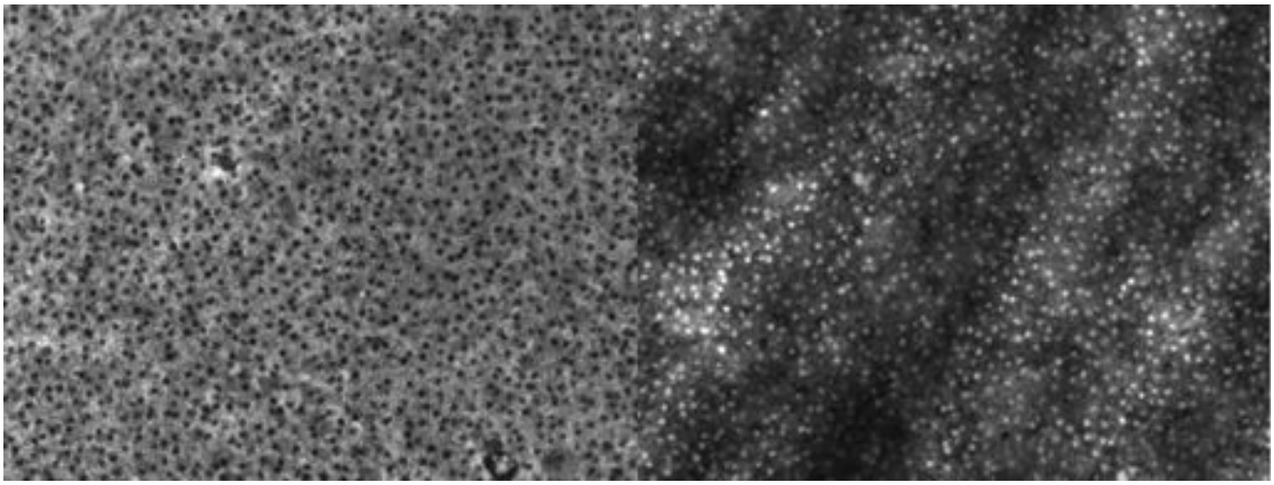


Figure 5: Left: Fluorescence donor retina microscope image. Right: Reflectance donor retina microscope image.

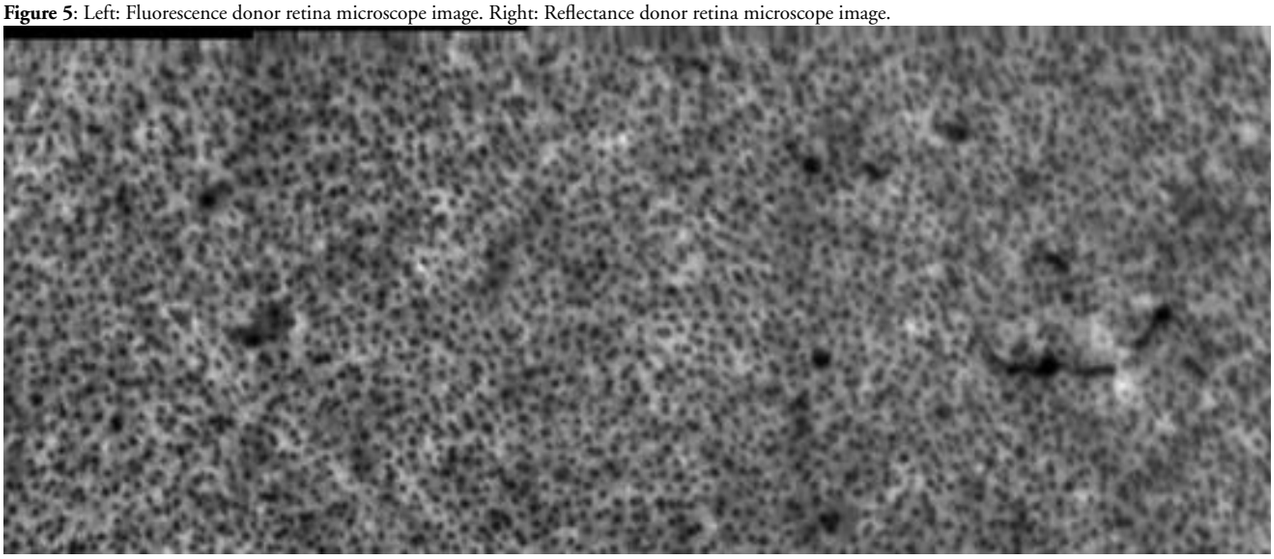


Figure 6: Fluorescence montage in a normal retina

Imaging individual RPE cells in primate retina in vivo

After imaging a cross-section of a donor retina using a conventional confocal microscope and seeing RPE cell features, we began to image a live primate retina to see the RPE cells in vivo. Primates are often used in experiments because they provide good models of the human eye for numerous experiments including testing treatments for retinal diseases.1 Additionally, in order to image a live primate, the primate is sedated so that the eye can be stabilized. We also used a peribulbar block1 to further minimize the amount of eye movements. Both fluorescence and reflectance images were then taken simultaneously. Figure 7 shows two registered fluorescence sums, one at the fovea and the other 10 degrees away. As a result of eye stabilization distinctive RPE cells were found. Looking at figure 7, one can see RPE cells in the shape of honeycombs. Furthermore, the RPE cells found in the fovea are smaller and flatter than the ones in the periphery.

Results from imaging individual RPE cells in normal human retina using AOSLO

Subsequent to imaging from a live primate retina and finding RPE cells, we wanted to find similar results from a normal human retina. However, imaging a primate retina is relatively easier than imaging human retina because of the fixation stability. One such factor is that under normal conditions, the image formed on the retina is never stationary. The eye is continually moving, but never shifts far from its mean position during maintained fixation. While imaging a human retina, the subject was required to hold their eye steady while staring at a beam of light. Due to blinking and involuntary eye movements, it was difficult to stabilize the eye completely, leading to shifts and distortions in both the fluorescence and reflectance movie files. To compensate for eye movements, a dual registration program was implemented.

After imaging using AOSLO, a dewarping program was used on both movies to correct for the sinusoidal horizontal scan. Results of a single frame from both movies are shown in Figure 8. After the movies have been dewarped, a general cross-correlation technique of image registration was used to average several frames in the two separate movie files. Through the cross correlation algorithm, frames are selected with little movement while frames with large displacements or low correlations are eliminated. This way, movement in the captured image series is compensated for, resulting in a sharper final image. The reflectance movie is used to calculate the cross correlation and estimate the motion, then this same estimation for motion is applied to the fluorescence movie. By simultaneously acquiring images using both reflectance and

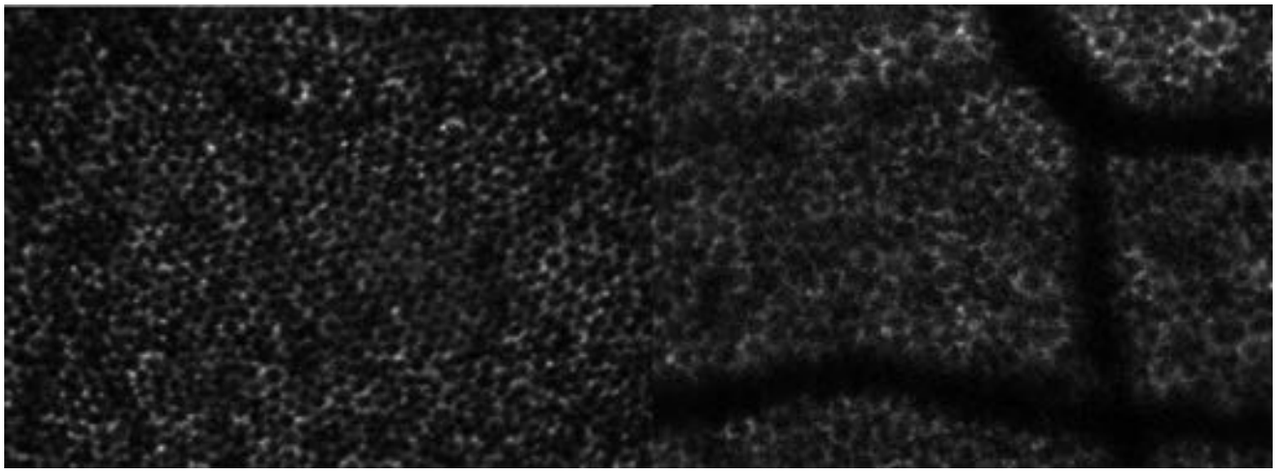


Figure 7: Left: RPE cells at the monkey fovea. Right: RPE cells at 10 degree eccentricity from the fovea. Scale: 1 pixel = 1μm

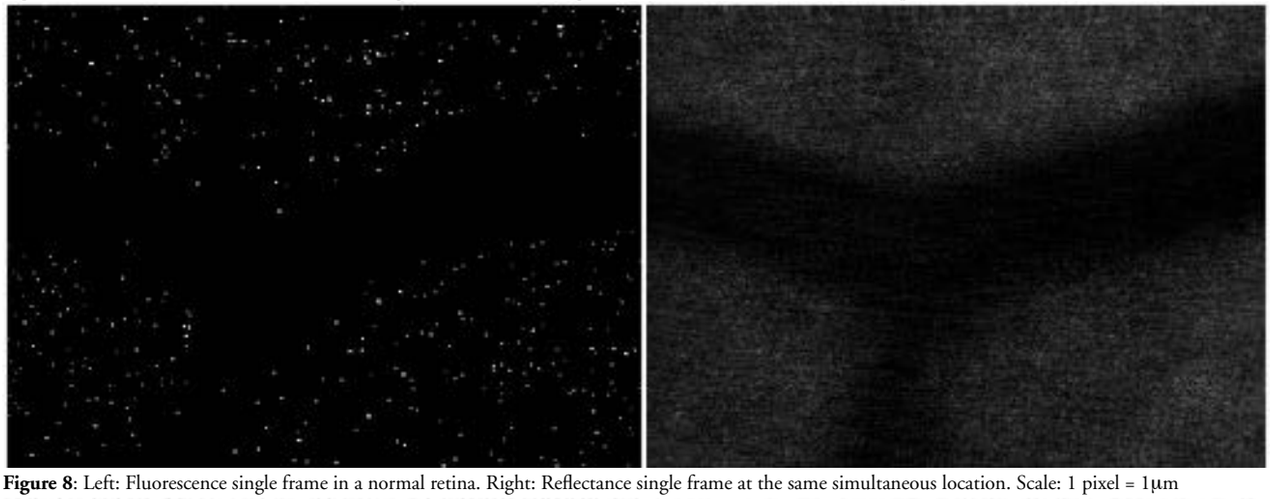


Figure 8: Left: Fluorescence single frame in a normal retina. Right: Reflectance single frame at the same simultaneous location. Scale: 1 pixel = 1μm

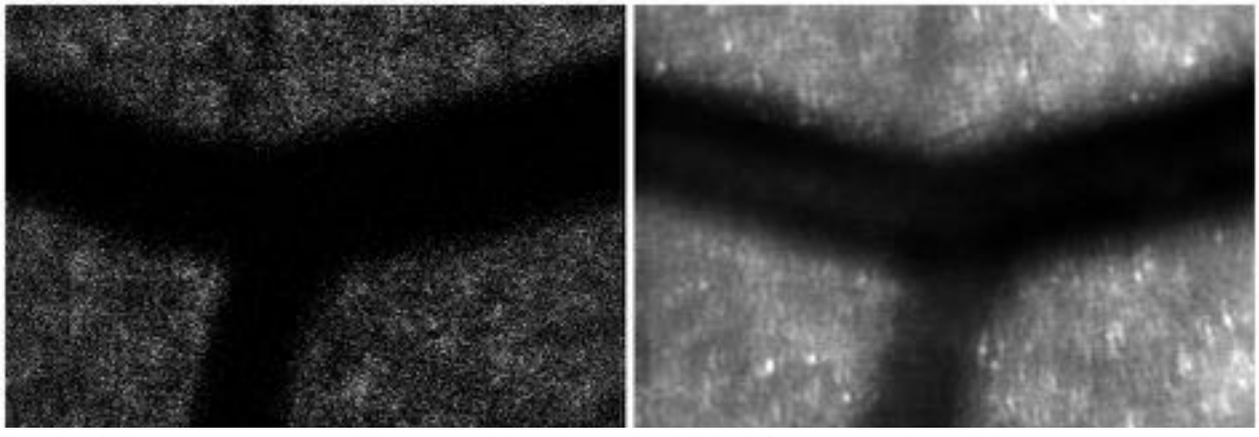


Figure 9: Left: Fluorescence registered sum of 564 images at 10 degrees Superior-Nasal. Right: Reflectance registered sum of 564 images at the same simultaneous location. Scale: 1 pixel = 1μm

fluorescence at the same retinal location, a high-contrast high resolution images are produced. This is seen below in Figure 9, a registered sum of 564 frames. Compared to the individual frames in Figure 8, images having gone through the dual imaging program showed clearer and distinct retinal features such the blood vessel and the RPE layer. However, individual RPE cells have not currently been resolved, additional improvements can be applied. One such technique would be to use a dual focusing system to correct for the eye's chromatic aberration and therefore to improve registration. With the dual focus, the two beams of light used for simultaneous imaging of reflectance and fluorescence can both be focused on retinal features despite the eye chromatic aberration. With this technique, the reflectance beam could be focused on the photoreceptor layer and the fluorescence beam focused on the RPE. Having a focused reflectance and fluorescence image simultaneously should lead to improved image registration. Eventually, with this improvement, individual RPE cells may be seen in a human retina.

Conclusion

Studying the RPE cells is important because they provide

critical support to the photoreceptors as well as help researchers understand the fundamental cause and progression of retinal diseases. They are also unique because they contain auto fluorescent properties that can be detected using adaptive optics. Although our abilities to characterize these cells were limited by many factors such as software programs and imaging techniques, our findings from imaging donor slides, monkey retina and human retina so far have contributed to the learning of RPE cell features. Future improvements such as using the dual focus will help eliminate the intra-frame motion to result in better image summation. Nevertheless, further research is required to help understand more about these RPE cells and to find ways for early detection and treatment of retinal diseases.

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About the Author

Katherine Shen received her BA in East Asian Studies and graduated in 2006 after completing a Take Five project in Italian Studies. She is currently now pursuing a joint O.D./M.S. degree with the possibility of continuing on for a Ph.D.

jur: What is your research about / what applications does it have?

The primarily part of my research was to understand more about RPE cells in the retina. These cells as have shown to be linked to macular degeneration, which is one of the leading causes for blindness. Using one of the latest imaging techniques, we were then able to image these cells in monkeys and humans.

jur: How did you become interested in this area of research? / What motivated you to do this research?

I took Visual Perception with Dr. Williams my sophomore year in college and the subject completely changed my focus on life. I became really interested in vision and its components because without them, our lives would be completely different. Then, my last semester of college, I decided to work with Dr. Williams and his Graduate students in hopes to understand more in depth of how one layer in the retina can lead to many ocular diseases.

jur: How does this research relate to your major/future plans/interests?

I had been interested in going to Optometry school since junior year and once I found out I was accepted, this research allowed me to focus more on the research part of the program. Thus, I am now pursuing a joint OD/MS degree with the possibility of continuing on for a PhD.

jur: While doing this research project, what was your biggest obstacle and how did you overcome it?

One of the hardest things was imaging the RPE cells. We had build an adaptive optics system with a scanning laser ophthalmoscope in hopes to see individual RPE cells clearly. This technique was still in the process when I was there so it took a long time to generate good data consistently. However, we were able to find a few really good images.

jur: After completing your project, what do you think was your most fulfilling experience?

The most rewarding experience was definitely understanding what it was like doing research. I was given my own project with deadlines that I had to fulfill. I attended lab meetings just as if I was a grad student and read numerous papers regarding the subject.

jur: Any advice you can give to fellow undergraduates who would like to do this kind of research? (or any other type of research)

Pick an area where you would be interested in learning more about. You don't have to know anything about the subject because that is what research is about. Also, pick a good advisor who would help you out step by step.

Political Culture in Colombia and Ecuador: An Insight into Economic Performance Evaluation and Free Trade Agreements

Michael Castro, 2007

Advised by Paula D. McClain, Ph.D. and Scott de Marchi, Ph.D.

Department of Political Science, Duke University

Public opinion in Latin America in relatively free democracies is the most accurate tool for identifying salient issues in society. The probability of signing Tratado de Libre Commercio, Free Trade Agreement, of the Andean countries of Colombia, Ecuador and Peru with the United States depends upon a micro-level analysis of the individuals in these countries. This analysis will examine how political ideology, ethnicity, education and income affect perceptions of future economic performance among Colombians and Ecuadorians. In Colombia and Ecuador, various societal norms account for the differences between both countries. This paper presents evidence supporting the notion that individuals with high levels of education and income expect a high economic future while Indigenous people and leftists expect their country's economic future to fare poorly. The internal composition of Colombia and Ecuador may shed some light on why Colombia has signed the TLC and why Ecuador is reluctant to sign the treaty.

Introduction

Colombia and Ecuador share a significant history together as former colonies of the Spanish crown accounting for many social, economic and political similarities. The dearth of authoritarian regimes in Colombia and Ecuador leaves both countries apart from the second wave of democracy in Latin America. Several factors attempt to explain why Colombia and Ecuador have been able to remain relatively free democracies. Adam Przerworski and Fernando Limongi found that as a country's GDP increases, the probability that it will transition from a democracy to a dictatorship is extremely low.[1] Countries enact economic reform to increase economic growth or address the issues of the people. The reasoning behind such reforms, e.g. neoliberalism, is the paternalistic status of governments over their constituents where the government's decisions are rarely contested.

The legitimacy of democratic regimes, especially in Latin America, is based upon the notion of descriptive and substantive representation. Consequently, the decisions deliberated by representatives who supposedly attain both characteristics needed for effective government, use this legitimacy to abuse their power. Free Trade Agreements in Colombia and Ecuador center upon regional economic integration in lieu of signing agreements with the United States. Most of the literature on political economy in Latin America analyzes the effects of free trade on the individual country's economy, economic integration and on political institutions.

The Free Trade Agreement of the Andean countries of Colombia, Ecuador and Peru, with the United States is intended to increase economic growth and foreign investment in these countries. Support and skepticism of this free trade agreement is coming from the most important sector in a democracy: the underprivileged. In order to fully understand a country's agenda, a micro-level (survey) analysis is needed to determine whether or not the people support certain initiatives that their government is trying to implement. In this analysis, Colombia's and Ecuador's probabilities of signing the Tratado de Libre Commercio (TLC) depend on individual evaluation of the economy's future in each respective country. Colombian and Ecuadorian political culture will help explain the dichotomy between the countries. Political ideology, race, education and income are examined to see if in fact these concepts are able to predict economic performance evaluation in Colombia and Ecuador. Furthermore, there is a difference between Colombia and Ecuador given the fact that as of 2006, Colombia has signed the free trade agreement, while Ecuador has not signed the treaty.

The Free Trade Agreement of the Americas signed in 1998 by most Latin American countries left the window open for various free trade agreements to develop in the following years. The manner in which these free trade agreements are implemented or even negotiated raises many questions about the oligarchies in power in Latin America and the United States. The dynamic of legitimate representation in Latin America in the past ten years has changed with the election of leftist presidents and legislators. Leftist are individuals with populist, anti-neoliberal, and possibly anti-U.S./International Monetary Fund economic and political policies. While Ecuador has followed this path, Colombia is the only current country in Latin America to elect a right-winged president, Alvaro Uribe, and re-elect him for a second term. Right-winged individuals support free-market economics, strong partnerships with the U.S. and policies that would attract foreign investment. Political ideology, thus, is a strong predictor of economic performance evaluation in both Colombia and Ecuador. If Colombians and Ecuadorians are

Independent Variables	Coefficient (Std. Error)
Ideology	-.01 (.005) *
Mestizo	-.005 (.031)
Indigenous	.143 (.070) **
Education	-.021 (.003)***
Country	.265 (.030) ***
Constant	2.293 (.058) ***
N observations	2934
Adj. R^2	.044

*** P-value ≤ 0.000.; ** P-value ≤ 0.05.; * P-value ≤ 0.10.
Table 1: OLS Regression of Ideology, Indigenousness, Education and Country and Future economic performance evaluation in Colombia and Ecuador.

strategic voters, they voted for their current president on a variety of dimensions to represent them to the fullest extent.

Indigenous groups have politically mobilized themselves strongly in Ecuador by forming powerful political parties as well as by ousting President Jamil Mahuad in early 2000.[2] The overwhelming predominance of Indigenous peoples in Ecuadorian politics, and Colombian politics to a lesser extent, needs to be taken into account to accurately predict future economic performance evaluation. Conventional predictors of future economic performance evaluation only explain certain aspects of Colombian and Ecuadorian life and do not demonstrate the reality in both countries. These predictors, such as GDP, are skewed by the few individuals who are exorbitantly wealthy and imply that most individuals have some form of income. For continuity purposes, education and income are used as predictors of future economic performance evaluation. This paper will indirectly describe the current sociopolitical climate in Colombia and Ecuador by analyzing future economic performance determined by political ideology, race with respect to Indigenous groups, education and income.

Literature Review

The focus of economic performance, or perceived economic forecast, in Latin America concentrates on individual perception of economic well-being. Carol Graham and Sandip Sukhtankar analyze the effect of economic downturn on support for free market economics.[3] Graham and Sandip find that as respondents' perception of economic well-being improves, their opinion of democracy and free market economics improves as well.[4] Economic performance evaluation, thus, elicits respondents to take several factors into account when evaluating their personal economic situation or that of their country. Graham and Sukhtankar claim the support for free market economics by conventional demographics and whether a country had an economic crisis. This only explains one aspect of how individuals form their evaluation of economic policies and the economy in general.[5] Furthermore, the nature of the question analyzed by Graham and Sukhtankar and most political economists in Latin America causes them to focus their analysis on macro-level issues.[6]

Multi-national survey analysis of support of free market and trade policies find that Colombians are more likely to support free market and trade policies than most Latin Americans.[7,8] The methods of both papers focus on the predictive power of education and type of employment on support for economic integration and free trade in Latin America. Respondents who are highly educated tend to have highly skilled jobs. Colombians, compared to other Latin Americans, have at least an opinion of and some knowledge about free trade economics.[9] Colombia, it seems, is more likely than most Latin American countries to sign a free trade agreement with the United States. The basis, however, on which Colombia has these sentiments, has yet to be explored empirically. On the other hand, Enzo Grilli argues that free market reforms had "weak political econom[ic] support" in Latin America. Ecuador falls under this school of political economy.[10] The introduction of free market, neoliberal reforms has mixed results in Latin America and has strong effects on democracies in there; at times, it results in the fall of a democratic regime to an authoritarian regime.[11]

Democracy in Colombia and Ecuador and their respective political cultures give some explanation on attitudes toward policy. The amount of literature regarding the effects of both political ideology and race, however, is very limited and generalized. In order to determine the effect of political ideology and race in Colombian and Ecuadorian political culture, a contemporary synopsis of the state of democracy is needed. Marta Lagos argues that the apprehensive attitudes towards democracy in most Latin American countries are being affected by political and economic crises.[12] Support for democracy has been decreasing in Colombia, while support for democracy in Ecuador fluctuates.[13] Additionally, in both Colombia and Ecuador, the trust in political institutions outside of the presidency has been decreasing, which raises the question of how and where Colombians and Ecuadorians come up with their opinions of politics.[14] The answer relies upon the analysis of political culture in both countries.

Juan P. Luna and Elizabeth J. Zechmeister study political representation in nine Latin American countries, Colombia and Ecuador being two of the nine, with respect to the extent to which political parties represent "voter's policy preferences".[15] Luna and Zechmeister, in analyzing this question, designate countries on a "conservative" scale determined by the political parties' ideologies and their respective policy initiatives.[16] Colombia is given a high conservative score while Ecuador is the least conservative country in their analysis. Thus, Colombia is more conservative or right leaning than Ecuador, which is more liberal or left leaning. Christian Bjornskov analyzes the effect of political ideology on economic growth and determines that "rightwing societies have grown faster in the last decades than other democratic societies."[17] Moreover, Bjornskov's methods further support the notion of Colombia as more conservative than Ecuador. This notion may explain why Colombia and Ecuador have different GDPs. Macro-economic level indicators, such as GDP, do not take into account the stark realities of both societies.

Paul Collier studies the "effects of ethnic diversity on economic performance" by distinguishing among democracies and dictatorships.[18] Collier's argument focuses on differentiating among democracies and dictatorships and their economic policies caused by ethnic diversity.[19] Countries with high levels of ethnic diversity are not able to implement efficient economic policies because they need to contend with developing economic policies that will benefit all ethnic groups. Consequently, dictatorships usually implement redistributive

economic policies in countries with high levels of ethnic diversity. Ethnicity, inferring from Collier's argument, has no effect on how a country evaluates its economy. This argument is not valid, however, in the Colombian and Ecuadorian context. Political mobilization of Indigenous peoples in Ecuador, and Colombia to a lesser extent, vilifies Collier's assumption of ethnicity not affecting economic growth and performance evaluation.

Raul Madrid argues that Indigenous peoples in both Colombia and Ecuador have contested their rights as citizens and have become a political force enriching democracy in both countries.[20] Violent political demonstrations, consequently, are reduced because Indigenous peoples have individuals to politically represent them in national issues. The formation of political parties increases the probability of political participation among Indigenous peoples. These political parties tend to be "leftist" groups which focus on grassroots, redistributive economic policies. Francisco Panizza finds that contemporary "leftist" political parties in Latin America are converging to the median voter by shifting their policies more to the center.[21] Indigenous peoples' political parties, thus, have a chance of surviving in the political sphere if they shift some of their policy initiatives to be somewhat more inclusive of others.

Marcela Velasco Jaramillo contends that neoliberal economic reform both reinforces legacy, social class and the separation of ethnicities in Colombia and Ecuador.[22] Colombia and Ecuador, however, have changed since the fragmentation of *La Gran Colombia*.[23] Colombians are more concerned with social class, evident in labor mobilization, regardless of ethnicity to some extent, and protests. On the other hand, Ecuadorians are more concerned with ethnicity, evident in Indigenous peoples' political mobilization. Jaramillo further examines Colombia and Ecuador by designating Colombia as a Mestizo country and Ecuador as not.[24] Political power, thus, in Colombia rests in the hands of high and middle income social classes, which is mostly comprised of whites and Mestizos. Indigenous peoples in Ecuador have more political clout than most Indigenous peoples in Latin America because Indigenous Ecuadorians have formed powerful political parties. Thus, a substantial amount of political power rests in the hands of Indigenous peoples in Ecuador. The dichotomy of political culture in Colombia and Ecuador is explained by different political ideologies, identities and mobilizations.

Table 2: OLS Regression of Ideology, Indigenousness and SES and Future Economic Performance Evaluation (country-level).

Independent Variables	Coefficient (Std. Error)	
	Colombia	Ecuador
Ideology	-.023 (.009) **	-.003 (.008)
Mestizo	.005 (.048)	-.009 (.047)
Indigenous	.178 (.100) *	.038 (.106)
Education	-.017 (.005)***	-.012 (.004)***
Income	-.039 (.014) ***	-.030 (.008) ***
Constant	2.487 (.094) ***	2.613 (.084) ***
N observations	1109	1583
Adj. R^2	.030	.018

*** P-value ≤ 0.000.; ** P-value ≤ 0.05.; * P-value ≤ 0.10.

Theory

Public opinion polls are the most adequate tools for political scientists to analyze political participation, attitudes and behavioral aspects in a country. Assessments of public opinion in Latin America focus on presidential approval, attitudes of democracy and ideology with less emphasis on economics. Contemporary analysis towards economics in public opinion polls in Latin America highlight two main factors, education and income, to scrutinize support of free trade initiatives, economic integration and economic performance evaluation.[25,26,27] These analyses concentrate on factors which emphasize the differences between Latin American countries. In order to fully understand economic public opinion in Colombia and Ecuador, it is necessary to conduct an in-depth analysis of issues which lead individuals to form their opinions.

The framework of analyzing economic public opinion on the basis of education and income levels does not indicate specific cultural and ideological factors endogenous to Colombians and Ecuadorians. Political ideology and ethnicity, in respect to being Indigenous, are imperative in studying political culture in Colombia and Ecuador on the basis of the historical, economic and cultural affinity of both countries. Unlike other former Spanish colonies, after independence, Colombia and Ecuador along with Panama, Peru and Venezuela formed *La Gran Colombia*; nevertheless, they instilled the colonial legacy of a hierarchical, privileged order of political actors hindering the stability of their democracies.[28] Furthermore, Jorrin and Martz identify three main characteristics of political ideology and cultural affinity in these Andean countries: 1) "sweeping social and economic change" (integration of the lower classes into the political process); 2) "democratic rule rather [than] authoritarian;" 3) "a movement that is strictly native to the area" (*indigenismo* or pride in being Indigenous).[29] Historically, "leftist" political ideology has its support from lower social classes and, in Latin America, it also has its support from non-whites. Thus, political ideology and ethnicity complement each other in Colombia and Ecuador. Additionally, economic integration, the probability of free trade with other countries, and the implementation of a capitalist economy are remnants of the colonial legacy.[30]

Analysis of free trade agreements concentrates on macroeconomics while ignoring the effects of these reforms on the plebiscite. Within Latin America, political economists focus on the effects of macro-level indicators of economic growth, GDP, trade deficits, etc., on political institutions. Economic performance evaluation is adequate in estimating an individual's opinion toward economic policy. Most individuals, however, do not make a holistic analysis of the economy. Most individuals make their decisions on salient issues. In Colombia and Ecuador, a few of these issues include unemployment, investment and the free trade agreement with the United States. These economic performance measures become important during election time and politicians utilize these measures to benefit them in the long run especially if they are incumbents with positive records. Most Latin Americans are retrospective voters who will vote for those candidates who perform well in office. Thus, utilizing future economic performance evaluation as an indicator of whether or not an individual supports the free trade agreement is appropriate. Differences between political cultures, ideology and ethnicity within Colombia and

Ecuador can be accounted for by the extent to which both countries implemented the different definitions of identity and mobilization of Indigenous people.

The separation of La Gran Colombia meant that Colombia and Ecuador would go down similar paths, but divergence would also mean different political outcomes. Mestizos (mixed European and Indigenous peoples) comprise the majority of the races in Colombia and Ecuador, hence, they are more powerful than Indigenous populations and descendants of Africans. More importantly, Mestizos are, for the most part, phenotypic white individuals. Using Leticia Heras Gomez's framework of assimilation, more Colombians, than Ecuadorians, self-classify themselves as whites or Mestizos, even though they may be in fact a member of an Indigenous group.[31] Ecuadorian Indigenous groups are more likely to be involved in political mobilization and protest, a fact that was evident in the ouster of President Jamil Mahuad in 2000. Jose Guadalupe Vargas Hernandez studies the probability of Indigenous peoples mobilizing as economic downturn in a country is increased by the implementation of neoliberal economic policies.[32] Since more individuals identify themselves as Indigenous peoples in Ecuador, the issue of ethnicity is imperative to fully understand political ideology and future economic performance evaluation. Most Indigenous peoples have leftist political ideologies because leftist ideologies are progressive and inclusive. It is up to the discretion, however, of these peoples to become politically mobilized. The concept of being left-winged or right-winged is affected by whether an individual considers themselves as being part of a racial group. Colombians identify themselves as whites or Mestizos more often than Ecuadorians, thus most Colombians identify themselves as right-winged individuals. Identity has an opposite effect among Ecuadorians when compared to Colombians. Bjornskov argues that right-winged societies value individualism more than left-winged societies.[33] Individualism, being a principle tenet of free-market economics, helps produce an informed, productive society whose economic performance measures are high. Individualism leads individuals to become more productive because it will ultimately mean higher wages. Thus, identity and political ideology affect each other in Colombia and Ecuador. In most democracies, right-winged individuals are more prone to support free market economics than left-winged individuals.[34]

Hypothesis

The subject of this analysis requires a basic hypothesis describing the relationship between future economic performance and ideology, ethnicity, education and income.

H_{TLC}: *Individuals in Colombia and Ecuador will have a favorable evaluation of their respective country's economic future if they identify themselves as Mestizos, are on the right of the political spectrum, have high levels of income and are well-educated.*

This theoretical framework sets up several hypotheses for both Colombia and Ecuador, since there are various similarities and differences between the two countries.

Hence, in Colombia, the hypothesized relationship is as follows:

$H_{COLOMBIA}$: *Respondents who classify on the right of the political spectrum will have a favorable evaluation of the country's economic future. Respondents who classify themselves as Mestizos will have a favorable evaluation of the country's economic future. Highly educated respondents will have a favorable evaluation of the country's economic future. Respondents with higher levels of income will have a favorable evaluation of the country's future.*

Meanwhile, Ecuador's hypothesized relationship is as follows:

$H_{ECUADOR}$: *Respondents who classify on the left of the political spectrum will have an unfavorable evaluation of the country's economic future. Respondents who classify themselves as Indigenous will have an unfavorable evaluation of the country's economic future. Highly educated respondents will have a favorable evaluation of the country's economic future. Respondents with higher levels of income will have a favorable evaluation of the country's future.*

Thus, differences in both countries elicit one last hypothesis:

$H_{EVALUATION}$: *Overall, there is a difference between the two countries: Colombians rate their country's economic future higher than Ecuadorians.*

Data and Measures

The data sets concerning Colombia and Ecuador were provided by Latin American Public Opinion Project of Vanderbilt University. For accuracy and continuity purposes, the chosen year of study is 2004 because this was the most recent data set available for both countries. The dependent variable and the independent variables are selected if they appear in both data sets. The data sets are merged for a multi-nation analysis and to accurately depict what is happening in both Colombia and Ecuador at the same time. Unfortunately, a variable measuring individuals' perception of free-trade agreements with the U.S. is lacking. The dependent variable chosen for this analysis is *econperformance* where the concept that is measured is "Do you think in the next 12 months the economic situation of the country will be: 1) Better; 2) Same; 3) Worse," scaled from better to worse. This variable is the most appropriate variable in measuring future economic performance evaluation and free-trade agreements, however, there is not much variation in the respondents answer as their answer is constrained by the nature of the question.

In order to measure the desired outcomes, several independent variables are chosen to test the hypotheses. *Ideology* is measured on a 10 point scale from liberal to conservative or as phrased in the question from the "left"-wing to the "right"-wing. The manner in which the question of ideology is measured by this variable means that most respondents should not be constrained. *Ethnicity* is recoded into two separate dummy variables, *mestizo* and *indigenous*, to accurately test the hypotheses. It is not logical to measure the concept of ethnicity without making sure the model takes both Mestizos and Indigenous peoples into account. *Education* is measured by the number of years of schooling the respondent has completed. *Income* is measured into categories of income levels.[35] A dummy variable *country* is created in order to analyze

the two countries as a whole. Under time constraints, OLS regression is used to analyze the relationship of the dependant variable and the independent variables.

The predicted model is describing the relationship of future economic performance evaluation to ideology, ethnicity (whether Mestizo or not and whether Indigenous or not), years of education, income range and what country the respondents are from. More importantly, the model distinguishes between Colombians and Ecuadorians by predicting that Colombians will have a positive view of their country's economic future while Ecuadorians have a negative view of their country's economic future. My OLS model of these relationships is below:

econperformance = a - b1 (ideology) - b2 (mestizo) + b3 (indigenous) - b4 (education) + b5(country).

Since two countriesare being analyzed, there are two sets of predictions orhypotheses reflecting the differences among both countries.The relationship or the prediction in Colombia elicits this model:

econperformance = a – b1 (ideology) – b2 (mestizo) + b3 (indigenous) – b4 (education) – b5 (income).

Colombian respondents who classify themselves on the "right" of the political spectrum will evaluate the country's future economic performance more favorably. Mestizos in Colombia will have a more favorable evaluation of their country's future economic performance, while Indigenous peoples will evaluate Colombia's future economic performance less favorably. As the number of years of education increase and income range increases, respondents' evaluation of Colombia's future economic performance increases. In Ecuador, meanwhile, I predict the same affect but with a different model:

econperformance = a - b1 *(ideology)* – b2 *(mestizo)* + b3 *(indigenous)* – b4 *(education)* – b5 *(income).*

Ecuadorian respondents, who classify themselves on the "left" of the political spectrum, will evaluate the country's future economic performance less favorably. Mestizos in Ecuador will have a more favorable future economic performance while Indigenous peoples will have a less favorable future economic performance evaluation. I expect the relationship of education and income to be the same in Ecuador as in Colombia.

Results and Analysis

I expect the multivariate OLS regression analysis of the dependent and independent variables to demonstrate a strong relationship between most of the independent variables and the dependent variable. The model of the actual regression analysis supports the hypothesized relationship of the independent variables to dependent variable:

econperformance = 2.293 - .01(ideology) - .005(mestizo) + .143(indigenous) - .021(education) + .265(country)

Indigenous and *country* are good predictors of future economic performance evaluation. Indigenous peoples will decrease future economic performance as predicted and is statistically significant at the .05 level thus allowing me to reject the null hypothesis in this case. Indigenous peoples have an affect on Colombia's and Ecuador's future economic performance evaluation; however, in reality Indigenous peoples in Colombia compose less than 5 percent of the population and in Ecuador they compose 25 percent of the population. The difference in both Colombia and Ecuador in terms of variable *country* is statistically significant at the .000 level. The OLS regression model supports the notion that Colombians rate their country's economic future higher than Ecuadorians.

Ideology, *mestizo* and *education* are weak predictors of future economic performance evaluation. As hypothesized, respondents who classify themselves on the left of the political spectrum have a less favorable evaluation of the economy's future while respondents who classify themselves on the right have a more favorable evaluation of the economy's future. While this relationship is not supported by the model, the direction of the coefficient and its statistical significance demonstrate it could be a strong predictor if there were no missing values. My variable for *ideology* is significant at the .10 level meaning that there is some statistically significant relationship between ideology and future economic performance evaluations. Future economic performance evaluation appears to increase as education increases and is statistically significant at the .000 level. The coefficient for *education*, however, demonstrates that education is not good predictors of future economic performance evaluation. Mestizos, however, have a favorable evaluation of economic future but, it is not statistically significant in this data set. An in-depth analysis takes into account the shortcomings of the model.

There are only three categories for my dependent variable; thus, the variance of the dependent variables will be small. This demonstrates the weak relationship of the independent variables explaining the dependent variable, even though most of the independent variables are statistically significant. Other forms of regression analysis would be more appropriate to analyze the relationship, but due to limited time and knowledge of statistical methods, they were not utilized. Other logical problems with the model are with dataset itself. Missing values for both the dependent variable and *ideology* independent variable affect the adjusted R^2 of the model. In total, missing values for the dependent variable are 626 values and for the *ideology* independent variable are 1,156 values.

Since Colombia and Ecuador are being analyzed in one data set, the differences within each country are not being accounted for but have implications in the political sphere. As stated earlier, Ecuador has a higher population of Indigenous peoples than Colombia, meaning that Indigenous peoples in Ecuador have more political clout because of sheer numbers and previous experiences with political mobilization. In addition, the income disparity between Colombia and Ecuador would also have an affect on future economic performance evaluations, which is not taken into account with this model. With more time, this model would take into account other variables tailored to both countries. Table 2 summarizes the effects of independent on the future economic performance evaluations of Colombia and Ecuador (country-level).

The model of the actual regression analysis for Colombia, for the most part, supports the hypothesized relationship of the

independent variables to the dependent variable:

econperformance = 2.487 – .023 (ideology) + .005 (mestizo) + .178 (iIndigenousIndigenous) – .017 (education) – .039 (income)

Ideology, *education* and *income* are good predictors of future economic performance evaluation in Colombia. Colombians who are on the "right" of the political spectrum do in fact have a high evaluation of the country's economic future and is statistically significant at the .05 level, allowing me to reject the null hypothesis. Indigenous peoples in Colombia have poor evaluation of the country's economic future and are somewhat significant at the .10 level. Colombians with high levels of education have favorable views on the country's economic future and is statistically significant at the .000 level. Colombians with high levels of incomes also have favorable views on the country's economic future and is statistically significant at the .000 level. Mestizos, in reality, do not have favorable evaluation of the economy's future, not supporting my hypothesis, and is not significant at a statistical level. In comparison to the main OLS regression model of this paper, the number of missing values in the Colombia analysis is not that large.

The regression model of Ecuador is not supporting the hypothesized relationship between the independent variables to dependent variable:

econperformance = 2.613 - .003 (ideology) – .009 (mestizo) + .038 (indigenous) – .012 (education) – .030 (income)

Socioeconomic indicators of *education* and *income* are both statistically significant at the .000 level. Unlike Colombia, the non-SES independent variables are not significant at all. *Ideology* and *econperformance* are correlated in negative direction, "left"-winged ideology having a less favorable evaluation of economic future, but, it is not statistically significant in Ecuador. The same finding holds true for Mestizos in Ecuador; my OLS model predicted direction of correlation but, it is not statistically significant. Since Ecuador has a higher population of Indigenous people, it would be fair to make predictions stating *indigenous* would be statistically significant, however, it is not the case in this OLS model regression. Issues that arise from both models are comparable to the OLS regression model summarized in Table 1.

In analyzing the individual countries, the hypotheses $H_{COLOMBIA}$ and $H_{ECUADOR}$ are not fully supported by the OLS regression models summarized in Table 2. Hypothesis $H_{COLOMBIA}$ accurately predicts all relationships with respect to *ideology*, *education* and *income*; however, *mestizo* is not significant. Indigenous peoples in Colombia seem to do most of the ethnic explanation. Hypothesis $H_{ECUADOR}$ is only supported when analyzing education and income whereas *ideology*, *mestizo* and *indigenous* are not significant. The cross-national OLS regression model, as a whole, supports hypotheses H_{TLC} and $H_{EVALUATION}$. *Ideology*, *indigenous*, *education* and *country* have some form of statistical significance thus, political ideology, Indigenous peoples, years of education and what country a respondent is from has a strong relationship with future economic performance evaluation.

Future economic performance evaluation is able to give some insight in the Free Trade Agreement with the United States. Using this empirical analysis, I suspect that Colombia has signed the treaty because of "right" wing ideology. Furthermore, Colombians who have high levels of education and income have a more favorable evaluation of their country's economic future. Overall, education and income are directly correlated with future economic performance evaluation. Political ideology and ethnicity are not taken into account in Latin American political economy, even though they are salient. Indigenous peoples' rights and necessities need to be addressed since the statistics demonstrate the importance of a politically mobilized ethnic group in Colombia and Ecuador. This analysis seems to provide some evidence that those Latin American countries with a large Mestizo population are more prone to introduce neoliberal, free market reforms.

Conclusion

The Free Trade Agreement of the Andean countries of Colombia, Ecuador and Peru is a salient issue in Andean politics. Future economic performance evaluation is a good indicator of the probability of a country signing a free trade agreement. This paper analyzed the relationship of political ideology, ethnicity, education and income affecting future economic performance evaluation in Colombia and Ecuador. Colombia and Ecuador serve as a framework for a micro-level analysis of political culture affecting political economy. The study of individual preferences is an appropriate measure of legitimacy in Latin America. Political ideology and ethnicity are very salient concepts that have political and economic implications in Colombia and Ecuador. Indigenous peoples in both countries are a political force that the oligarchies in power have taken their acquiescence for granted. Further analysis of political culture among other Latin American countries will give answers to many unanswered questions. A cross-national analysis of political ideology is needed to determine the extent that "left"-winged ideology has on Latin America.

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Variable	Observations	Mean	Standard Deviation	Min	Max
Country	4,479	0.67	0.470	0	1
Econperformance	3,853	2.20	0.759	1	3
Ideology	3,323	5.82	2.392	1	10
Mestizo	4,479	0.69	0.464	0	1
Indigenous	4,479	0.04	0.206	0	1
Education	4,472	9.63	4.406	0	18

Cross-National LAPOP 2004 Data (Colombia and Ecuador)

LAPOP 2004: Colombia

Variable	Observations	Mean	Standard Deviation	Min	Max
Econperformance	1,403	2.02	0.797	1	3
Ideology	1,276	6.57	2.518	1	10
Mestizo	1,479	0.51	0.500	0	1
Indigenous	1,479	0.06	0.235	0	1
Education	1,478	9.41	4.646	0	18
Income	1,337	5.64	2.245	0	13

LAPOP 2004: Ecuador

Variable	Observations	Mean	Standard Deviation	Min	Max
Econperformance	2,484	2.27	0.726	1	3
Ideology	2,122	5.35	2.221	1	10
Mestizo	2,923	0.77	0.418	0	1
Indigenous	2,923	0.03	0.178	0	1
Education	2,991	10.21	4.281	0	18
Income	1,337	3.60	1.799	0	10

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34. Ibid.
35. The dataset of both countries demonstrate there are different categories which impedes an OLS regression analysis of income. Furthermore, the currencies for both Colombia and Ecuador are different adding more error to the OLS regression model if income is added. The Colombian Peso is converted into US $ but, the categories in the Colombian data set do not

correspond to the categories in Ecuadorian data set which use US $ as their currency. More importantly, introducing PPP(Purchasing Power Parity) will increase the errors in the model because there is no logical manner in which to reconcile the differences. Income is analyzed in the individual countries.

About the Author

Michael Castro graduated in 2007 with a degree in Political Science and Linguistics.

jur: What is your research about ? What applications does it have?

My research is about how individuals form opinions about economic policies and reform in Latin America. Free Trade Agreements with the US are the most salient economic policy that Latin American countries are enacting. Conventional predictors of support neoliberal economic policies are unable to explain why certain countries implement these policies. My research supports the notion that race and political ideology are important in determining support for neoliberal economic policies.

jur: How did you become interested in this area of research? What motivated you to do this research?

I became interested in this area of Latin American Politics because I wanted to see why some countries like Colombia signed the Free Trade Agreement with the US. Ecuador, however, is reluctant to sign the treaty. I wanted to explore the dynamics of popular support for economic policies.

jur: How does this research relate to your major/future plans/interests?

I plan to pursue a Ph.D. in Political Science focusing on Latin American Racial/ Ethnic Politics, Political Ideology and Public Opinion.

jur: While doing this research project, what was your biggest obstacle and how did you overcome it?

My biggest obstacle in this research project was attaining the dataset with the public opinion data that I needed. I was fortunate enough to have Duke's full resources available and I was able to continue with my research.

jur: After completing your project, what do you think was your most fulfilling experience?

The most fulfilling experience after completing my research was that I was granted the opportunity to present the paper at the American Political Science Association Annual Meeting 2006 in Philadelphia.

jur: Any advice you can give to fellow undergraduates who would like to do this kind of research?

My advice to individuals who want to pursue any type of research is to develop a research plan, talk to professors with similar interests and stay focused.

Mindfulness and its Moderating Effect on the Relation of Religiosity to Psychological Health

Matthew Della Porta, 2006

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Religion is a large point of interest in our society. Its dominant influence throughout history and current presence in the media has led to both positive and negative stereotypes about the psychological health of those who are religious.[1] Consequently, researchers have investigated the relation of religiosity to psychological health. The underlying dimensions of religiosity and the mediating or moderating roles of other variables have been examined.

Religiosity

Meta-analyses of the research on the relationship between religiosity and psychological health have been conducted, although they have yielded inconsistent results. Wong, Rew, and Slaikeu (2006) found that 90% of the 20 studies conducted between 1998 and 2004 showed that high levels of religiosity are associated with better mental health in adolescents.[2]

In contrast, Bergin (1991) concluded in his meta-analysis that there was no relation between religiosity and mental illness or better mental health. However, he explained that this may have been due to studies that did not differentiate between separate dimensions of religiosity.[3] Ryan, Rigby, and King (1993) support this notion by asserting that religiosity can only be related to psychological health when we consider in what way a person is religious.[4] Similarly, Hackney and Sanders (2003) found that researchers find different results depending on the way that they define religiosity.[5] Therefore, in order to fully explore religiosity and its relation to psychological health, it is necessary to examine its underlying dimensions.

Koenig, Parkerson, and Meador (1997) claim that there are three commonly accepted dimensions of religiosity: organizational, nonorganizational, and subjective (intrinsic). Organizational religiosity refers to attendance at religious meetings such as a church or synagogue. Non-organizational religiosity refers to engagement in independent religious activities such as prayer or meditation. Subjective religiosity indicates the extent to which a person incorporates his or her religious beliefs into daily life.[6]

All three dimensions of religiosity have been simultaneously linked to psychological health. Frazier, Mintz, and Mobley (2005) and Levin, Chatters, and Taylor (1995) found all three dimensions of religiosity to be related to several measures of psychological well-being in an African American sample.[7,8] Religiosity as a whole has also been shown to be unrelated to psychological distress.[9]

Organizational religiosity has been linked to fewer depressive symptoms and higher positive affect,[10] as well as linked with an improvement and maintenance of good mental health.[11] Nonorganizational religiosity has been found to be a mediator between religiosity and psychological well-being.[12] Subjective religiosity has been shown to be positively related to psychological adjustment, relative to people who are non-religious.[13]

There is also evidence suggesting that the three dimensions of religiosity are not all related to psychological health. Storch, Storch, and Welsh (2002) found that only subjective religiosity was negatively correlated to symptoms of depression.[14] Also, Dezutter, Soenens, and Hutsebaut (2006) found that subjective religiosity predicted well-being, while the organizational dimension did not.[15]

The role of a third variable in the relation between religiosity and psychological health has also been explored. Meaning in life was found to be a mediator between religiosity and several positive psychological outcomes (Steger & Frazier, 2005).[16] In addition, Tix and Frazier (2005) found personal strivings to be mediators in the negative relation between intrinsic religiosity and hostility, and also found religious tradition to be a moderator of the relation between intrinsic religiosity and both anxiety and depression.[17] In this research, the possibility of a third variable in the relation between religiosity and psychological health has been supported.

To summarize, researchers have not agreed on which, if any, dimensions of religiosity are related to psychological health and which aspects of psychological health are associated with religiosity.[18] Most importantly, the mediating or moderating roles in this relationship of other variables have not yet been adequately investigated. Mindfulness is a variable that could play an important role in the relationship between religiosity and psychological health.

Mindfulness

Mindfulness is the purposeful and non-judgmental focus of attention and awareness on the present moment (Kabat-Zinn, 1994). The practice of mindfulness meditation is a fundamental teaching of Buddhism, but it is also practiced both by those with other religious orientations and by those who practice no religion. Kabat-Zinn (1994) stated that "mindfulness has little to do with religion, except in the most fundamental meaning of the word, as an attempt to appreciate the deep mystery of being alive and to acknowledge being vitally connected to all that exists" (p. 6).[19] In this manner, mindfulness may have a role in the effect of religiosity on psychological health. It is a characteristic that can be appreciated by and applied to any religious orientation.

Empirical evidence supports the apparent positive nature of mindfulness by association with positive psychological outcomes. Brown and Ryan (2003) found that mindfulness promotes both well-being and self-regulated behavior.[20] Also, advanced practitioners of mindfulness meditation reported greater positive mood than beginning practitioners.[21] Finally, mindfulness has also been related to increased activity in the left anterior portion of the brain, which has been associated with a positive affect.[22]

Religiosity, in conjunction with mindfulness, may be associated with psychological health. Specifically, the inherent positive characteristics of mindfulness and its relation to self-regulated behavior facilitate an optimal religious practice that is deep, purposeful, and satisfying.[20] Such a practice will likely be associated with positive psychological outcomes. In contrast, a religious practice without mindfulness will leave a "void" of unawareness and a lack of self-regulated behavior in religious experience. This will be associated with religious activities and beliefs that are automatic, without true intent, and ultimately unsatisfying. Therefore, religiosity will be less strongly related to psychological health when it is not accompanied by mindfulness.

Exploration of the moderating role of mindfulness may explain the contradictory findings of past research on religiosity. Steger and Frazier (2005) and Tix and Frazier (2005) have already demonstrated the critical role that a third variable may play in the relation of religiosity to psychological health, and mindfulness is an ideal variable to test.[16,17] Its link to well-being and self-regulated behavior has already been demonstrated[20] and the positive inherent characteristics of mindfulness, such as awareness and attention to the present moment, make it a plausible factor in the relation between religiosity and psychological health. Mindfulness will be related to an ideal religious practice that will be accompanied by psychological health.

I hypothesize that mindfulness will have a moderating effect on the relationship between religiosity and psychological health (i.e., religiosity will be related to psychological health only when it is accompanied by a high level of mindfulness). Once the moderating effect of mindfulness has been explored, the relationship between religiosity and psychological health can be further elucidated.

Method

Participants

Participants were 132 undergraduate students (84 women, 34 men, 14 who did not indicate gender). The average age was 20.25 (*SD* = 1.56) and ranged between 18 and 25. The majority of the sample identified themselves as "White" (75.0%), while the rest were "Asian American or Pacific Islander" (3.8%), "Latino" (3.8%), "African American" (3.8%), "Other" (3.0%), and 14.0% who did not indicate ethnicity. The predominant religious affiliation of the sample was Christianity (58.3%), while the rest did not actively practice a religion (35.6%), were Jewish (3.0%), or practiced a religion other than Christianity or Judaism (3.0%). Participants completed an online survey that lasted approximately thirty minutes in exchange for extra credit in a psychology course. Participants were recruited through a psychology research bulletin sign-up.

Materials

Mindfulness. The Mindful Attention Awareness Scale (MAAS)[20] assessed trait levels of mindfulness (15 items; e.g., It seems I am "running on automatic," without much awareness of what I'm doing). Responses were made on a 6-point Likert-type scale, ranging from 1 (almost always) to 6 (almost never). The reliability was $\alpha = .94$.

Religiosity. The Duke Religion Index (DRI)[6] measured three dimensions of religiosity. The first was organizational religiosity (1 item; i.e., How often do you attend church, synagogue, or other religious meetings?). Responses were made on a 6-point Likert-type scale, ranging from 1 (never) to 6 (more than once a week). The second dimension was nonorganizational religiosity (1 item; i.e., How often do you spend time in private religious activities, such as prayer, meditation or Bible study?). Responses were made on a 6-point Likert-type scale, ranging from 1 (rarely or never) to 6 (more than once a day). The third dimension was subjective or intrinsic religiosity (3 items; e.g., My religious beliefs are what really lie behind my whole approach to life). Responses were made on a 5-point Likert-type scale, ranging from 1 (definitely not true) to 5 (definitely true). The reliability was $\alpha = .75$.

Mood. The Positive and Negative Affect Scale (PANAS)[23] measured positive (10 items; e.g., excited) and negative (10 items; e.g., scared) affect. Responses were made on a 7-point Likert-type scale, ranging from 0 (never) to 6 (extremely). The reliability for both positive and negative affect was $\alpha = .87$.

Life Satisfaction. The Temporal Satisfaction with Life Scale (TSWLS)[24] measured life satisfaction in the past (5 items; e.g., If I had my past to live over, I would change nothing), present (5 items; e.g., I would change nothing about my current life), and future (5 items; e.g., There will be nothing that I will want to change about my future). Responses were made on a 7-point Likert-type scale, ranging from 1 (strongly disagree) to 7 (strongly agree). The reliability for each subscale ranged from $\alpha = .91$ to .93.

Subjective Vitality. The Subjective Vitality Scale (SV)[25] was used to assess vitality (7 items; e.g., I feel alive and vital). Responses were made on a 7-point Likert-type scale, ranging from 1 (strongly disagree) to 7 (strongly agree). The reliability ranged from $\alpha = .84$ to .86

Self-esteem. The Rosenberg Self-esteem Scale (SES)[26] was used to measure self-esteem (10 items; e.g., I feel that I am a person of worth, at least on an equal basis with others). Responses were made on a 4-point Likert-type scale, ranging from 1 (strongly disagree) to 4 (strongly agree). The reliability was $\alpha = .77$.

Self-Actualization. The Self-Actualization Index (SAI)[27] was used to measure self-actualization (25 items; e.g., I am loved because I give love). Responses were made on a 7-point Likert-type scale, ranging from 1 (strongly disagree) to 7 (strongly agree). The reliability was $\alpha = .65$.

Depression. The Center for Epidemiological Studies Depression Scale (CES-D)[28] was used to measure depressive symptomatology (6 items; e.g., I feel sad). Responses were made on a 7-point Likert-type scale, ranging from 1 (not at all) to 7 (very much). The reliability ranged from $\alpha = .84$ to .85.

Anxiety. The Profile of Mood States Scale (POMS)[29] was used to measure anxiety (9 items; e.g., Tense). Responses were made on a 5-point Likert-type scale, ranging from 0 (not at all) to 4 (extremely). The reliability ranged from $\alpha = .63$ to .96.

Results

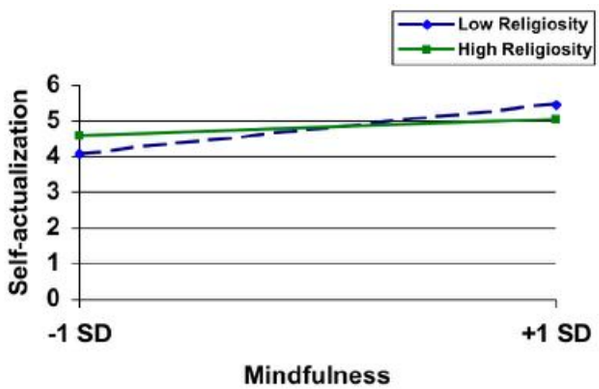
Out of 132, 10 participants were excluded from analysis due to insufficient data.

Hierarchical regression was used to analyze the main and interaction effects of religiosity and mindfulness on psychological health. The religiosity and mindfulness variables were first centered, and the interaction term was created as the product of these two variables.[30]

Anxiety. Using hierarchical regression, anxiety was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, F (2, 112) = 15.24, $p < .01$, $\Delta R^2 = .21$. Although religiosity was not related to anxiety ($\beta = -.03$, *ns*), mindfulness was negatively related to anxiety ($\beta = -.46$, $p < .01$). However, in step 2 of the regression analysis, the interaction term was not a significant predictor ($\beta = .00$, *ns*). The interaction effect was F (1, 111) = 0.00, *ns*, $\Delta R^2 = .00$.

Self-actualization. Using hierarchical regression, self-actualization was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, F (2, 109) = 13.17, $p < .01$, $\Delta R^2 = .20$. Although religiosity was not related to self-actualization ($\beta = .04$, *ns*), mindfulness was positively related to self-actualization ($\beta = .44$, $p < .01$). In step 2 of the regression analysis, the interaction term was a significant predictor ($\beta = -.19$, $p < .05$). The interaction effect was F (1, 108) = 4.75, $p < .05$, $\Delta R^2 = .03$. Those both low and high in religiosity were higher in self-actualization as their level of mindfulness increased.

Figure 1: Interaction between self-actualization and mindfulness.



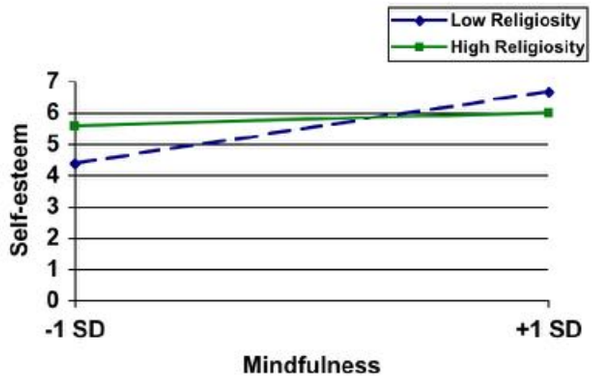


Figure 2: Interaction between self-esteem and mindfulness.

The simple slopes of this interaction, plotted at +/- 1 standard deviation of mindfulness, appear in Figure 1.

Self-esteem. Using hierarchical regression, self-esteem was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, F (2, 111) = 13.92, $p < .01$, $\Delta R^2 = .20$. Although religiosity was not related to self-esteem ($\beta = .14$, *ns*), mindfulness was positively related to self-esteem ($\beta = .41$, $p < .01$). In step 2 of the regression analysis, the interaction term was a significant predictor ($\beta = -.23$, $p < .01$). The interaction effect was F (1, 110) = 7.91, $p < .01$, $\Delta R^2 = .05$. Those both low and high in religiosity were higher in self-esteem as their level of mindfulness increased. The simple slopes of this interaction, plotted at +/- 1 standard deviation of mindfulness, appear in Figure 2.

Subjective Vitality. Using hierarchical regression, subjective vitality was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, F (2, 114) = 13.13, $p < .01$, $\Delta R^2 = .19$. Religiosity was positively related to subjective vitality ($\beta = .18$, $p < .05$) and mindfulness was positively related to subjective vitality ($\beta = .38$, $p < .01$). In step 2 of the regression analysis, the interaction term was a significant predictor ($\beta = -.17$, $p < .05$). The interaction effect was F (1, 113) = 4.33, $p < .05$, $\Delta R^2 = .03$. This interaction was slightly less pronounced than the interactions for self-actualization and self-esteem. When the level of mindfulness increased, those high in religiosity showed a higher level of subjective vitality than those low in religiosity. The simple slopes of this interaction, plotted at +/- 1 standard deviation of mindfulness, appear in Figure 3.

Depression. Using hierarchical regression, depression was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, F (2, 112) = 16.46, $p < .01$, $\Delta R^2 = .23$. Religiosity was negatively related to depression ($\beta = -.18$, $p < .05$) and mindfulness was negatively related to depression ($\beta = -.43$, $p < .01$). In step 2 of the regression analysis, the interaction term was a significant predictor ($\beta = .23$, $p < .01$). The interaction effect was F(1, 111) = 8.00, $p < .01$, $\Delta R^2 = .05$. Those high in religiosity were lower in depression as their level of mindfulness increased. However, those low in religiosity were more depressed as their level of mindfulness increased. The simple slopes of this interaction, plotted at +/- 1 standard deviation of mindfulness, appear in Figure 4.

Life Satisfaction. Using hierarchical regression, life satisfaction

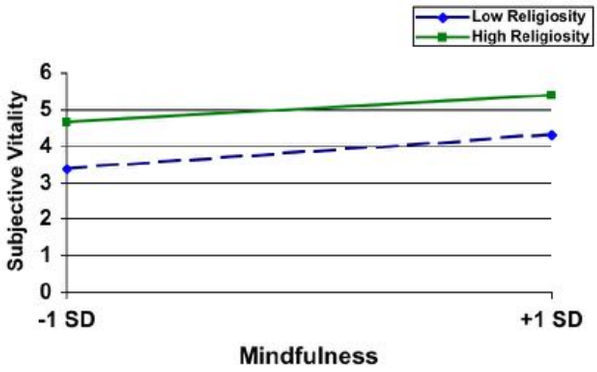


Figure 3: Interaction between subjective vitality and mindfulness.

was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, $F(2,106) = 9.93$, $p < .01$, $\Delta R^2 = .16$. Religiosity was related to life satisfaction ($\beta = .19$, $p < .05$) and mindfulness was positively related to life satisfaction ($\beta = .33$, $p < .01$). However, in step 2 of the regression analysis, the interaction term was not a significant predictor ($\beta = -.15$, *ns*). The interaction effect was $F(1, 105) = 2.75$, *ns*, $\Delta R^2 = .02$.

Positive Affect. Using hierarchical regression, positive affect was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, $F(2,115) = 7.70$, $p < .01$, $\Delta R^2 = .12$. Religiosity was not related to positive affect ($\beta = .14$, *ns*) and mindfulness was positively related to positive affect ($\beta = .31$, $p < .01$). However, in step 2 of the regression analysis, the interaction term was not a significant predictor ($\beta = -.05$, *ns*). The interaction effect was $F(1, 114) = .35$, *ns*, $\Delta R^2 = .00$.

Negative Affect. Using hierarchical regression, positive affect was regressed onto the religiosity and mindfulness variables in step 1. The overall model for this step was significant, $F(2,115) = 14.57$, $p < .01$, $\Delta R^2 = .20$. Religiosity was not related to negative affect ($\beta = -.03$, *ns*) and mindfulness was positively related to negative affect ($\beta = -.45$, $p < .01$). However, in step 2 of the regression analysis, the interaction term was not a significant predictor ($\beta = .05$, *ns*). The interaction effect was $F(1, 114) = .43$, *ns*, $\Delta R^2 = .00$.

Discussion

The moderating effect of mindfulness on the relation between religiosity and psychological health was explored. Significant interactions were found for self-actualization, self-esteem, subjective vitality, and depression. For those high in religiosity, mindfulness was positively related to self-actualization, self-esteem, and subjective vitality, and was negatively related to depression. For those low in religiosity, mindfulness was positively related to self-actualization, self-esteem, and subjective vitality, but was positively related to depression. As a result of the moderating effect of mindfulness, the religious practice of those high in religiosity was optimized and was accompanied by psychological health.

In addition, mindfulness was positively related to all of the positive indicators of psychological health: self-actualization, self-esteem, subjective vitality, life satisfaction, and positive affect. It was also negatively related to all of the negative indicators of psychological health, including anxiety, depression, and negative affect. Religiosity was negatively related to depression and positively related to subjective vitality and life satisfaction.

The interaction of both low religiosity and mindfulness on depression was unexpected. Depression may have been positively related to mindfulness for those with low religiosity because they are more intensely aware of their feelings and surroundings. This intensified awareness may have brought attention to unpleasant feelings, memories, or present experiences, resulting in emotional conflict. With no religious practice or beliefs to help resolve this conflict, levels of depression may have increased. Mindfulness has inherently positive qualities, but may make an individual more aware of unpleasantness that would not be as troublesome to a less mindful individual.[19]

Another limitation of this study is the use of an online survey. The participants were not in a controlled environment while completing the survey and could have been distracted, or they may not have completed the survey in one session. An ideal setting for the use of an online survey would be in a controlled environment. This may sacrifice the convenience with which participants could access the survey, but would offer more reliable data.

The results of this study raise several questions that could be investigated in future research. For instance, mindfulness and religiosity did not have a significant interaction effect on anxiety, life satisfaction, positive affect, or negative affect. Future research could attempt to replicate these findings in an effort to determine if the relations between religiosity and these particular measures of psychological health persist without being moderated by mindfulness. If such replication effects occur, the nature of these measures of psychological health could be further investigated. Ultimately, a distinction could be made between the measures of psychological health that have interaction effects and the measures that do not. This distinction could explain why such interaction effects do or do not occur.

The present study used a college undergraduate sample that was almost entirely either Christian or non-religious. These results could be found in other Christian samples, but the moderating role of mindfulness in the relation between religiosity and psychological health should also be explored in those with other religious orientations.

A point of interest could be Buddhism, which offers practices of mindfulness meditation as a fundamental teaching.[31] In a

Figure 4: Interaction between depression and mindfulness.

Buddhist sample, it is likely that religiosity and mindfulness would be positively related. High religiosity in other religious orientations is less clearly related to mindfulness. Based on the present findings, Buddhism, in comparison to other religions, would be most strongly related to psychological health because of its inherent association with mindfulness.

The present results also raise the question of whether there are differences in psychological health between participants high in mindfulness and those either low or high in religiosity. In a future study, two mindful samples (one religious and one non-religious) could be examined in order to determine if higher levels of psychological health are associated with those who are mindful and religious or with those who are only mindful.

Finally, the correlational nature of this study still leaves the question of the causality between mindfulness and psychological health unanswered. An experimental study using a mindfulness priming effect (such as a meditation instruction session) may be able to further explain this matter. In addition, a longitudinal study tracking the psychological health of a participant learning to meditate could further elucidate the nature of this relationship. Clinical studies using Mindfulness Based Stress Reduction (MBSR) already support this notion. Carlson and Garland (2005) reported reductions in sleep disturbance, stress, mood disturbance, and fatigue for cancer outpatients throughout an 8-week MBSR program.[32] A non-clinical population may also report positive psychological outcomes when learning mindfulness meditation over a period of time.

There has been a great deal of research done on the clinical applications of mindfulness and how it can reduce negative symptoms.[33] However, relatively little research has been done exploring mindfulness and its relationship to positive psychological outcomes. This study indicates the importance of mindfulness on one's psychological health both on its own and in relation to religiosity. The present results can encourage other researchers to explore mindfulness not merely as a clinical treatment, but also as a crucial component of psychological health for all individuals.

Future research could explore mindfulness and its relationship to other positive psychological variables such as compassion, openness to experience, honesty, and authenticity. Just as religiosity is considered to have sub-components, future research on mindfulness should consider a multi-faceted approach in order to most accurately and effectively discover its relationship to other positive variables.[34]

The present findings suggest the moderating role of mindfulness as a way to understand the relation between religiosity and psychological health. The empirical study of mindfulness is still in its infancy,[20] and there is still much to be learned from it. Empirical data on mindfulness will facilitate an accurate and appreciative mainstream understanding of its potential for psychological health. Indeed, the Buddha once said, "there is one road, one path for beings to purify themselves, to transcend sorrow and grief, to overcome suffering and melancholy, to attain the right way, to realize nirv_na: that is the . . . establishment of mindfulness."[31]

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About the Author

Matthew D. Della Porta graduated from the University of Rochester in 2006 and was awarded high honors in research in psychology. He plans on obtaining his Ph.D. in social/personality psychology and to work as a faculty member at a college or university. His research interests include many facets of positive psychology such as mindfulness, long-term positive affect, and creativity.

jur: What is your research about? What applications does it have?

My research concerns the role of mindfulness in optimal psychological health. Jon Kabat-Zinn defines mindfulness as the purposeful and nonjudgmental focus of attention and awareness on the present moment. In this case, I am examining mindfulness as a variable that moderates the relationship between religiosity and psychological health. This is just one example of the research being conducted exploring the important role of mindfulness in optimal psychological health. Such research can bring further attention to the importance of being focused on the present moment and to the practice of mindfulness meditation.

jur: How did you become interested in this area of research? What motivated you to do this research?

The notion of mindfulness can be traced back to many sources in Eastern religion and philosophy, which has been an area of my personal interest for many years. Learning about mindfulness offered a drastically different approach to everyday living compared to the relatively frantic pace of modern society. I feel that mindfulness has a tremendous potential to improve and enrich our daily lives. Research provides empirical support to demonstrate this potential and exposes mindfulness to other researchers and the general public.

jur: How does this research relate to your major, future plans, and interests?

One focus of my future research will be concerned with optimal psychological health. I will always consider mindfulness an important factor in this empirical initiative.

jur: While doing this research project, what was your biggest obstacle and how did you overcome it?

Completion of this research project went very smoothly. Perhaps the only serious obstacle in the completion of this project was making sure that I had enough participants. I was able to ensure this by making a constant effort to advertise my survey and by using the online system Sona to recruit participants from the U of R's psychology courses.

jur: After completing your project, what do you think was your most fulfilling experience?

The most fulfilling experience of completing this project was having the opportunity to present my results to the faculty of the department of psychology. The audience for my presentation was filled with outstanding researchers and it was truly an honor to be able to show them what I did and to answer their questions about my study.

jur: Do you have any advice you can give to fellow undergraduates who would like to do this kind of research, or any research in general?

For the conduction of research in general, my best advice is to stay obsessively organized and to make backup copies of everything. This project took months of work and I quickly learned that all of that work could go to waste if I wasn't very careful with all of the files I was working with. I would also say that it is extremely important to have an interest in your area of research that goes beyond mere curiosity. That enduring interest and passion in your work will keep you motivated throughout the arduous months that it takes to successfully complete a research project.

Peer Victimization: The Role of Self-efficacy in Children's Coping Strategies

Stefanie Putter, 2007

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Peer victimization, whether in the form of conflict, aggression, violence or bullying behaviors, is a common problem in today's schools systems.[1] Until recently, the severity and frequency of these issues were greatly underestimated. On a global level, researchers have estimated that between 7% and 23% of school-aged children are victimized by their peers.[2] Studies have also shown that one in six Australian students are bullied on a weekly basis and a further 77% of secondary schools students report being the victims of bullying at some point throughout their educational experience.[3, 4]

Consistently, studies have also shown that children who are victimized, whether in the form of harassment, ridicule, rejection or social isolation, are at a higher risk for social and psychological maladjustment. Specifically, victimized students have been reported to have a variety of health problems, including anxiety, depression, low self-esteem and negative affect.[5, 6, 7] Thus, from current research it is possible to conclude that victimized individuals often suffer physically, mentally and emotionally. Evidence has further revealed that these problems, which can interfere with the healthy development of young children, may persist throughout the school years and even into adulthood.[8]

It is apparent that peer victimization affects children to varying degrees. Hoover, Oliver and Hazler studied school children who were victims of bullying and discovered that each of these children were impacted differently from these experiences.[4] While over 75% of secondary school students reported being victimized throughout their educational careers, only 15% believed they had been affected in a detrimental manner. Consequently, researchers have started to focus on the adaptation of diverse coping strategies in order to explain differences in victimized children's adjustment.[7]

Coping has been defined as 'the way people manage life conditions that are stressful' (Lazarus, 1999, p.102).[9a] Coping strategies help to decrease the rate of victimization and mitigate the negative effects of bullying experiences. Depending on the coping strategies that children adopt in stressful situations, they might be more or less at risk of being victimized and of experiencing negative consequences from these bullying behaviours.[10]

Although most of the current research focuses on how often children utilize coping methods to deal with bullying situations, there is a lack of research assessing children's beliefs in their ability to use these strategies. This perceived capability in managing one's personal ability to function in stressful situations is referred to as "self-efficacy."[11] An increasing quantity of research is beginning to emphasize children's self-efficacy beliefs and the subsequent impact on peer and social influences and developmental outcomes.[12] It is this belief in one's ability to gain control during negative experiences that may help to reduce the risk of victimization and psychological maladjustment. Thus, if children do not believe in their ability to utilize particular coping strategies, they will be unlikely to use them effectively, if at all.

Researchers have commonly examined two different types of coping strategies: approach and avoidance. Approach strategies refer to direct efforts aimed at modifying the stressful situation, while avoidance strategies focus on managing and controlling cognitive and emotional responses to the situation. Examples of approach coping in a bullying context include problem solving, assertiveness and seeking social support. When a child independently chooses a means for reducing distress, this process is referred to as problem solving. Assertiveness, a problem solving technique, is a means of actively modifying the situation by standing up for one's self.[13] On the other hand, when a child turns to others for advice or assistance with the situation, a social support method is utilized.[14] Another approach strategy is conflict resolution, which is aimed directly at stopping victimization.[7] Overall, problem solving, seeking social support, conflict resolution and assertiveness are approach strategies that have all been associated with positive outcomes during victimization experiences.

In contrast to approach coping, avoidance strategies include processes such as internalizing and externalizing. Blaming oneself and worrying over the event are referred to as internalizing coping methods, while expressing negative emotions through overt reactions to other people or things are referred to as externalizing coping strategies.[15] Forgivingness, a process which often replaces these negative emotions with positive or other-oriented emotions, is another construct that has had little research, but would be interesting to examine in a bullying context.[16] On the other hand, an additional avoidance strategy that literature has shown to be positively

associated with peer victimization is revenge seeking.[7] This study aims to determine if avoiding revenge seeking behaviours may be correlated with lower levels of distress from bullying interactions.

In a study by Kochenderfer-Ladd and Skinner, children's coping strategies were analysed as possible moderators of the effects of bullying on children's adjustment. Analyses revealed that beneficial adjustment for victimized and non-victimized children depended on the adaptation of specific coping strategies. For example, problem solving strategies appeared to be effective for non-victimized children by helping them stay away from problems, whereas this coping style enhanced problems for victimized children who did not believe they were capable of dealing with the situation themselves. Furthermore, internalizing strategies were related to a higher risk for social difficulties and psychological maladjustment for victimized children. Thus, victims who did not blame themselves appeared to be buffered from social problems.[17] Although little support for externalizing coping methods has been evidenced for boys, Chung and Asher found that girls who utilize externalizing coping processes in stressful situations, through antagonistic or coercive tactics, are often rejected by their peers and poorly adjusted to their school environments.[18] The current study will investigate children's self-efficacy of coping strategies in terms of avoidance of both internalizing, externalizing and revenge seeking strategies and the adaptation of problem solving, support seeking, conflict resolution and assertiveness skills.

In order to select either an approach or avoidance coping method, it is important to make an appraisal of the stressful situation. Process theories of coping emphasise the role of one's perceptions or of the degree of control one has over the stressful situation, which can be construed as a measure of efficacy. Research has shown that appraisals of the stressor are important determinants of what coping style one chooses to employ.[19] A study by Hunter, Boyle and Warden examined the appraisal of coping processes used by 830 school children between nine and fourteen years of age in Scotland. Results demonstrated that children were more willing to seek help (approach coping style) when they believed they had some control of the stressful situation.[20] Thus, individuals who are able to construe victimisation in a more positive manner are more likely to seek help and thus reduce the risk of psychological maladjustment. The current study will also analyse children's ability to use coping strategies in terms of victim disengagement (i.e. not taking on the victim role; not taking other victimization personally) and control.

In particular, the current study will focus on children's self-efficacy for using different coping strategies. This study will examine an array of coping processes that have been identified from the intervention literature, including problem solving, seeking social support, conflict resolution, assertiveness, avoiding revenge, avoiding externalizing, avoiding internalizing, forgivingness, construal of victimisation, victim disengagement and control. In order to investigate how these different coping strategies interrelate with one another, it is necessary to perform a factor analysis. A factor analysis of the previously mentioned strategies will be used to establish the psychometric components of a newly developed coping scale. Although past research has demonstrated that the ability to gain control during a negative interaction is an important indicator of whether or not a child will adopt an approach or avoidance coping style,[12] a factor analysis will help determine if more specific coping strategies emerge for bullying victims. Overall, the current study will analyse children's propensity to use different coping strategies during peer victimization interactions. The aim of this study is to devise a better understanding of useful coping strategies for bullying victims which can then be used as a future guide for intervention programs.

Method

Participants

Parental consent to participate in the present investigation was obtained for 1375 children (719 boys and 656 girls) who attended Independent and Catholic schools in the Eastern, Western and Northern suburbs of Sydney, Australia. These participants were recruited from several grade levels to obtain a sample that was representative of secondary school-aged children (11 to 15 year-olds): grade 7 (boys= 251, girls= 210), grade 8 (boys= 234, girls= 232) and grade 9 (boys=234, girls= 214). Parents of all participants were sent letters explaining the aims of the study and were provided with either a passive or an active consent form, depending on the school administration's policy. For the passive consent forms, parents returned the forms only if they did not want their child to participate. On the other hand, active consent forms required parental signatures for student participation in the study. Verbal assent was also obtained from all those students who wished to participate in the study.

Procedure

Postgraduate students and research assistants from Macquarie University administered questionnaires to secondary school-children in the spring (term 1) of the school year. Participation consisted of a fifty-minute session which was supervised by student researchers and some teachers. The first five minutes of the session were spent giving directions and explaining that the aim of the study was to obtain student feedback on peer interactions. During group administrations, children were spaced adequately apart to ensure their answers would be kept private and confidential. The survey was divided into two sections, consisting of demographic questions assessing age, gender and ethnicity and a series of self-report questions.

Measures

For this study, a large coping efficacy scale was developed. Most of the measures were drawn from published articles while others were specifically developed for this project. The items created for this study were modelled after pre-existing scales, but re-worded as efficacy measures to fit within the bullying context. The items that were selected to go into this coping efficacy scale are discussed below.

Self-Report Coping Measure[15]: This measure was designed to examine coping strategies based on both the approach and avoidance conceptualizations.[21] For the purposes of this study, problem solving and seeking social support were the only approach strategies included, and for the avoidance strategies, only internalizing and externalizing were explored. Typical problem solving questions include "trying to think of different ways to fix the problem", while support seeking questions ask how well one can "get help from a family member". Examples

of avoiding internalizing and externalizing questions were "avoid crying about it" and "avoid yelling to let off steam". A total of four approach and avoidance strategies were assessed using this scale.

Assertiveness Scale[13]: Assertiveness was assessed as an approach strategy that is used to directly modify the situation by standing up for one's self. Examples of items include "in a calm and pleasant manner, tell the kid to stop" and "say something to stick up for yourself".

Conflict Resolution and Revenge Seeking Scales[7]: These measures assessed coping strategies of conflict resolution and revenge seeking in terms of various things children could do if they were being picked on. Children were asked if they 'would definitely do that' (3.00), 'maybe do that' (2.00) or 'no, would not do that' (1.00). Typical conflict resolution items included 'make a plan to get along with the kid who was picking on you' and 'take some time to cool off before responding'. Examples of avoidant revenge items included "stay calm and avoid wanting to hurt the kid in some way" and "avoid thinking about getting even with the kid".

Trait Forgivingness Scale[16]: Measures a respondent's self-appraisal of his or her proneness to forgive interpersonal transgressions. Sample items include "forgive and forget them picking on you" and "forgive them even if they have hurt you".

Victim Disengagement Scale: This measure was created for the purposes of this study in order to assess the victim's ability to not take on the victim role and to not take other victimization personally. This scale was designed to measure how some children are not bothered by peer victimization. Typical victim disengagement items include "keep from taking it personally by thinking, I don't care what they think anyway" and "stop yourself from taking it personally by thinking, I don't value their opinion".

Victim Construal Scales[20,22, 23]: The avoiding negative construal (or self-blame) scale developed for the current study assesses how well victimized children can avoid blaming themselves and feeling guilty for the things that they did or did not do. Sample avoiding negative construal questions include "avoid thinking, I am the one to blame for this" and "keep from thinking, why do they only pick on me". On the other hand, positive construal of victimization include questions like "being picked on will help me change or grow as a person in a good way" and "I will learn something positive from being picked on".

Control Scale[15]: This scale focused on children's self-efficacy of degree of control in peer victimization interactions. Sample questions include children's belief in their ability to control a situation where they had been "left out" and "hit, kicked or pushed".

Overall, the coping measures created for this study included 56 items divided into several categories: Problem Solving (3), Seeking Social Support (6), Avoiding Internalizing (3), Avoiding Externalizing (4), Assertiveness (2), Conflict Resolution (3), Avoiding Revenge Seeking (3), Forgivingness (3), Victim Disengagement (5), Avoiding Negative Construal (10), Positive Construal (4) and Control (9).

Results

Different coping strategies that children adopt during bullying interactions were analysed using a factor analysis procedure. Principal-components factor analyses with oblimin rotation resulted in the identification of six factors that accounted for 50% of the variance. An oblimin rotation method was utilized because the different coping strategies were expected to be correlated. For this study, the data fit very well into a six-factor model which accounted for a high level of the variance. Any items that loaded below 0.3 were dropped from analysis. In addition, two items were omitted because of cross-loading on multiple factors for which it was conceptually difficult to deem one factor a better fit than another. Factor loadings for the remaining items are reported in Table 1.

The first factor consisted of items predominantly relating to avoidant coping strategies, such as externalizing, revenge seeking and forgivingness. (i.e., 'avoid holding a grudge against them', 'avoid thinking about getting even with the kid'). High scores indicate high coping self-efficacy and this factor was labelled 'Avoiding Behaviours'. Additionally, the alpha value of factor 1 was calculated in order to check the reliability and was found to be 0.905.

The second factor consisted of items relating to the ability to gain control during negative interactions (i.e., making the situation better when you have been 'left out' or 'teased'). High scores indicate high coping self-efficacy and this factor was labelled 'Reconstructing'. The alpha value for this factor was 0.960.

Markers of the third factor related to avoiding self-blame during negative interactions (i.e., 'avoid thinking, I get picked on because of the way look', 'keep from thinking, it only happens to me'). High scores indicate high coping self-efficacy and this factor was labelled 'Avoiding Negative Construal' with an alpha of 0.901.

The fourth factor consisted of four items predominantly related to positive construal of victimisation (i.e., 'being picked on will help me become more self-confident', 'I will learn something positive from being picked on'). These four items had an alpha value of 0.883 and the factor was labelled 'Positive Construal'.

The fifth factor consisted of items demonstrating an approach coping strategy to bullying interactions (i.e., 'get help from a family member', 'try extra hard to keep it from happening again'). These items included processes of support seeking, problem solving and conflict resolution. This factor was labelled as 'Approach Coping' and had an alpha value of 0.853.

Finally, the sixth factor consisted of items related to avoiding worrying and taking peer victimization personally (i.e., 'avoid crying about it', 'stop your self from taking it personally by thinking, I don't care what they think anyway'). This final factor was labelled 'Victim Disengagement' and had an alpha value of 0.901.

Discussion

This study aimed to create a measure of children's self-efficacy for using various coping techniques. This study developed a psychometrically sound measure of children's beliefs in their abilities to use these strategies. Factor analysis of the entire scale revealed six main factors: Avoiding Behaviours, Reconstructing, Avoiding Negative Construal, Positive Construal, Approach Coping and Victim Disengagement.

Factor 1 consisted of several avoidant coping strategies that children believe they can adopt during bullying interactions. These included externalizing, revenge seeking, forgivingness, conflict resolution and assertiveness. Prior research has linked externalizing and revenge seeking together, demonstrating that using these strategies in stressful interactions can often lead to poorer social adjustment.[7, 15] Forgivingness, a process of replacing negative emotions with more positive ones, has been minimally researched.[16] Future research will be helpful to determine if this variable can be classified as an avoidant coping method. Additionally, conflict resolution and assertiveness are both typically viewed as approach strategies, but appeared within this factor as well.[7,13] While both of these variables had low factor loadings (0.473 and 0.467 respectively), further research is necessary to clarify these findings.

Factor 2 consisted of the variables found within the control scale that was devised for this study. These items included three different types of control: verbal, physical and relational. Together, all of these items correspond to ways in which an individual can attempt to make a stressful situation better. Past research validates these associations.[15]

Factor 3 included items assessing the victim construal of the situation. Based on past research by Graham and Juvonen, the avoiding negative construal items assessed how often children can avoid blaming themselves when being bullied.[23] Also included in this factor was an item that assessed avoidant internalizing behaviour. This item, "avoid becoming too upset to talk to anyone", is very similar to the avoiding negative construal items with an appraisal of one's self rather than the situation. While past research has not linked these two items together, avoiding a negative appraisal of the situation and one's self appear to be useful strategies that children are able to adopt during peer victimization interactions.[19]

Factor 4 consisted of variables associated with a positive construal of bullying interactions. Researchers have demonstrated that children who are able to construe victimization in a more positive manner are more likely to seek help and thus reduce the risk of psychological maladjustment.[22]

While Factor 1 included several avoidant coping strategies, Factor 5 included approach coping strategies that children believe they can adopt during bullying interactions. These included seeking social support, problem solving, assertiveness and conflict resolution. All of these techniques aim to modify the situation by making an active effort to talk to someone else, stand up for one's self, or change the situation. Past research findings have demonstrated a link between all four of these approach coping strategies and more positive outcomes during victimization experiences.[7, 13, 14]

Finally, Factor 6 consisted of variables associated with victim disengagement. A victim disengagement scale was created for the purposes of this study in order to assess the victim's ability to not take on the victim role and to not take victimization personally. Avoiding internalizing items were also included in this factor, as they were believed to be an additional method for measuring how some children are not bothered by peer victimization. Additional research on these variables is needed to assess the validity of these constructs.

While most of the findings in this study were highly significant, the study also had its limitations. First of all, one limitation of this study is that the data were only assessed at one time interval. In order to validate the effectiveness of children's coping strategies, longitudinal research is necessary. This plans to be completed later on this year, by collecting data from Term 4 of the 2006 school year from the same students tested in Term 1. Another limitation is the sample used for participants. Bullying behaviours appear to begin when children are as a young as five to six years old[2] and thus gathering data from a wider age distribution will either validate or help to improve the current findings. Furthermore, this study only assessed a selective number of possible strategies. There may be other more effective coping strategies for reducing psychomaladjustment following bullying that were not analysed in this study. Since only fifty percent of the variance was accounted for by the variables utilized in this analysis, it is important to look at additional variables that may play a significant role in peer victimization interactions.

The coping strategies analyzed in this study were used to examine children's self-efficacy in peer victimization interactions. Through a factor analysis of the chosen variables, six factors emerged as coping strategies that children believed would be effective during bullying interactions. This study appears to demonstrate that self-efficacy plays a large role in the effectiveness of coping mechanisms. Coping techniques usually associated with better outcomes in this study include avoiding revenge seeking, avoiding externalizing and internalizing, forgivingness, gaining control, avoiding negative construal, engaging in positive construal, assertiveness, problem-solving, seeking social support, conflict resolution and avoiding taking it personally. Thus, if children can be trained to use these coping strategies efficiently, it may be possible to further reduce victimization and psychological maladjustment. Future research should investigate how children's self-efficacy in various coping strategies varies with age and gender. Different findings may be evident as a result of these additional variables. Furthermore, future analyses should assess more specifically how the adaptation of coping strategies is shaped by children's belief in their ability to use these measures effectively. The model will need to test all of these variables in order to establish validity as these beliefs may be related to the rate of victimization (i.e., whether the bullying behaviours have continued or ceased) and the degree of psychomaladjustment the child experiences.

The coping strategies analysed in this study have high alphas and explain fifty percent of the variance, indicating that these techniques should have some predictability. Overall, more research is necessary to establish whether children with a greater ability to use these strategies are associated with less victimization. Future research will need to assess the validity of such conceptualizations.

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About the Author

Stefanie Putter will receive her B.A. Psychology from the University of Rochester in May 2007. She plans to ???

jur: Is there a particular area or aspect of your major that you wanted to further explore with this current research? Or, did you purposely choose this topic because it wasn't as related to your direct area of your study?

I am so fortunate to have been given the opportunity to participate in psychological research in Sydney, Australia. While I have been a research assistant under the direction of several faculty members at the University of Rochester, going abroad and participating in research was an entirely new experience. Working on this particular project I was able to fully engage in all aspects of the psychology research process, from gathering and entering data, to running statistical analyses and finally constructing a journal article based on the findings of a subset of the data.

jur: How did you arrive at this research topic in the first place?

As an intern in the developmental psychology department, I worked with a postgraduate student, Puneet Singh, as she began to gather data for her doctoral dissertation. The main focus of her thesis regards peer victimization and the coping strategies that school children utilize to effectively deal with these situations. I analyzed a subset of the data that was collected, as her project was only in the beginning stages, and focused my paper on the self-efficacy of children°Øs coping strategies.

jur: How did you go about refining your focus for this research?

At the time, no analyses had been done on the gathered research and I had to option of choosing what subset of the data I would like to focus on. I chose to examine coping self-efficacy as a means of performing difficult statistical analyses, including a factor analysis which I have never done before.

jur: Initially, what was your main goal for this research—what did you ultimately want to achieve?

Throughout the creation of this journal article, my main goal was to further learn about the research process. As a result of this internship experience, I was able to expand my understanding of statistics, refine my writing abilities and learn in-depth knowledge about Australian school children.

jur: Provided the above question, was there any point in your research where your main objective changed, and, how did that affect the outcome of the research?

I believe that everything I have learned from this experience will greatly aid my future psychological research endeavors. As an active participant in the research process, I am able to continually refine my skills and knowledge of the psychology field.

jur: Is your currently completed research a topic that you will be exploring further?

While I do not intend to continue research on this particular project, it is possible that I may investigate related areas in the future. At the current moment I am not participating in any research pertaining to peer victimization; however, I feel that this is an interesting area of study that I may decide to pursue one day.

jur: What was most difficult about getting the research ready and together? What aspects, do you think, were easiest?

One of the most challenging aspects of this particular research study was actually gathering the data. In Australia, it was very difficult to get approval by the school districts to allow students to participate in psychological research. Eventually, we got approval to gather data from several Independent and Catholic schools in suburbs of Sydney. I have definitely realized the necessity for patience and determination to succeed in psychological research. Setbacks are always going to occur, so it is important to persist and focus on the end goal.

One of the easiest aspects of the research process was working with the graduate students and my supervisor, Dr. Kay Bussey. They provided an extremely supportive environment which made me feel comfortable to ask questions and inquire further about various aspects of the research project.

jur: How do you think getting published will help your research, and, what kind of impact (if any) do you want to have on colleagues by getting this published?

While I never intended to get this article published, I believe that it is important to show others, especially those interested in psychology research, that there are no boundaries in this field. Even as an undergraduate, there is the potential to study cross-cultural research. While I am just beginning my research career, I have already established a psychological network at the University of Rochester and at Macquarie University in Sydney, Australia. As a result of my research internship in Australia, I definitely have a greater appreciation for psychology researchers and I hope that one day I can contribute to this continually evolving field.

A Brief Overview of Emerging Nanoelectronics

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Current semiconductor technology is projected to reach its physical limit in about a decade.[1] To satisfy the ever-growing consumer desire for small, high-speed, low-power, and "all-in-one" devices, scientists and engineers are actively seeking solutions to continue technology trends into the future. Nanotechnology, with wide electronics applications, promises breakthroughs for the future. The fundamental principles of nanotechnology lie in nanoscience, the study of materials and phenomena at atomic scales, where macroscopic properties are no longer relevant. Nanotechnology is thus the application of nanoscience, encompassing physical, chemical and biological systems at scales up to 100 nanometers (nm), as well as integration of nanostructures into macroscopic systems.[2] Nanotechnology covers a wide range of science and engineering fields, but its current active areas of research and development can be divided into four groups—nanomaterials, nanometrology, nanoelectronics, and bio-nanotechnology. Nanomaterials have structured components with at least one dimension at the nanometer scale;[2] for example, nanoparticles are considered three-dimensional nanomaterials. Nanometrology is the science of measurement at nanometer scales. It involves the measurement and characterization of nanomaterials (i.e. size, shape, properties, etc.) as well as development of tools for measurement and characterization. It is a crucial area because it facilitates nanotechnology research and sets the standard for industrial practice. Bio-nanotechnology deals with biophysical, biochemical and biomedical mechanisms, properties, and applications at molecular scales. This paper focuses on nanoelectronics, the application of nanotechnology in electronics. Specifically, carbon nanotubes, nanowires, and quantum dots (nanocrystals) are discussed, because of their great potential for electronic systems.[2]

Basic Concepts

The energy band model, ballistic electron transport, and field-effect transistors are introduced here to aid the reader with the discussions in the next sections.

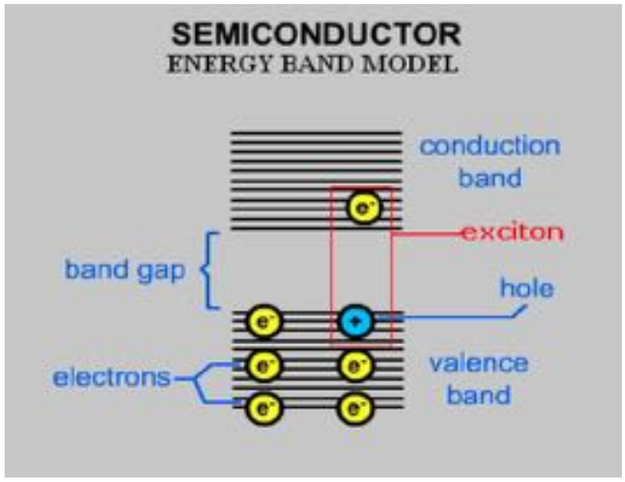
Energy Band Model

Electrons in semiconductors have a range of discrete energies. Based on the Bohr model, the electrons in an atom are limited to certain energies, which are restricted to quantized values. Hence, the electrons can be visualized as traveling in well-defined orbits, each of which may contain different numbers of electrons, representing quantized energy levels.[3] The separation between adjacent energy levels is extremely small that they can be considered a continuous band. The *bandgap* can then be pictured as a region between continuous flow of energy bands not occupied by any electrons (Fig. 1). The size of the bandgap varies depending on the material. Electrons occupying energy levels below the bandgap are described as being in the *valence band*, while those electrons occupying energy levels above the bandgap are said to be in the *conduction band*. The electrons are able to move freely in the conduction band and conduct electricity. In semiconductor materials, the majority of electrons are confined in the valence band while there are very few occupying energy levels in the conduction band. Electrons, however, can jump across the bandgap from the valence band into the conduction band when given enough energy from external stimuli, such as thermal excitation, voltage, and photon flux, leaving in the valence band some vacancies known as "holes", which are considered to be positively charged. The raised electron and the hole as a pair are called an *exciton*.[3]

Ballistic Electron Transport

Ballistic electron transport is the phenomenon of electrons traveling from one electrode to another without scattering (Fig. 2a). It is introduced here because in one-dimensional

Figure 1: Energy Band Model (Source: Evident Technologies, Inc.[43])



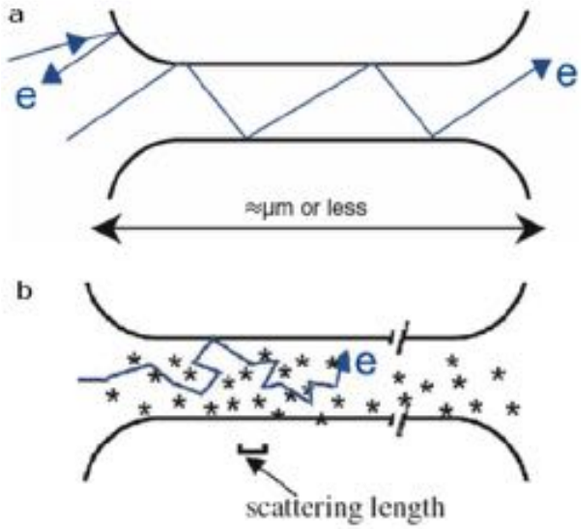


Figure 2a: Ballistic electron transport. Electrons are only backscattered at the boundary. **Figure 2b:** Diffusive Electron Transport. Numerous scattering events are visible. (Source: A.M. Song[5])

nanostructures, which will be discussed later, the electron transport is often observed to be ballistic. One important concept leading to ballistic electron transport is the *electron mean free path* (l_e), which is known as the average distance electrons can travel ballistically before encountering any random scatterers.[5] The significance of this concept is that, when the device is miniaturized to scales equal to or smaller than the mean free path, electron transport in the conduction channel becomes ballistic. When the transport is not ballistic, it is termed diffusive; in this regime, electrons traveling in the conduction channel encounter numerous scattering events (Fig. 2b). In contrast to ballistic transport, diffusive transport can often be observed at macroscopic scales. However, under ballistic transport, where electrons are often only backscattered at the boundaries of the conduction channel, the concept of ohmic resistance is no longer relevant.[5] Because of the small scale dimensions at which ballistic transport occurs, quantum confinement effects play a significant role, and therefore conductance G of the material can be defined by the Landauer-Buttiker formula:[7,8,9]

$$G = N(2e^2/h)T \qquad (1)$$

where e is the electron charge, h is Planck's constant, N is the number of the occupied quantum confinement modes by electrons in the channel, and T is the transmission coefficient of the ballistic electrons going from one electrode to another.[5] One of the advantages that ballistic transport offers is higher operating speeds, since electrons do not encounter random scattering in the channel. Because of the absence of scattering, fewer electrons are required in the channel to pass the signal, leading to less power consumption.

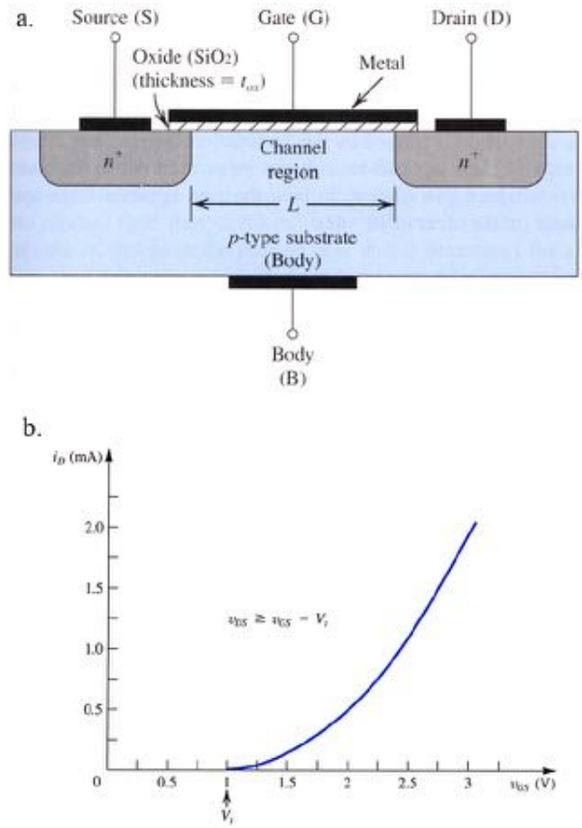
Field-effect Transistors (FETs)

FETs are fundamental building blocks commonly found in modern semiconductor electronics. They are devices that have three electrode terminals known as *source*, *drain* and *gate*. The current can flow in between the source and drain through the *channel*, with the gate controlling the current flow. The channel is insulated from the gate to prevent electrons from tunneling through the gate (Fig. 3a). There are n-type and p-type FETs. In the n-type device, the current will start flowing from the source to drain when a positive voltage, greater than a threshold V_t, is applied to the gate (Fig. 3b); in the p-type the current flows when the gate voltage is negative.

Carbon Nanotubes

Carbon nanotubes (CNTs), as the name suggests, are tubes with diameters in nanometer (nm). A CNT can be pictured as a honeycomb-structured sheet of graphene, which is a single atomic layer of graphite, wrapped up to form a cylindrical tube. The CNTs can be open on both ends, or bounded in one or both ends. There are two main types of CNTs—single-walled nanotubes (SWNT) and multi-walled nanotubes (MWNT). The MWNTs are several concentric SWNTs of different diameters nested together. Typical diameters of carbon nanotubes range from 1 nm to 5 nm. However, the length of the nanotubes can range from hundreds of nanometers to several centimeters long. Researchers at the New Jersey Institute of Technology have reported self-assembled 10-foot-long hollow thin steel tubing SWNT.[26] This extremely large length-to-diameter ratio makes the CNTs perhaps the most ideal one-dimensional structure in real world practice.[1] In contrast, given the same length, objects like regular metal wires may have much larger diameters and therefore are commonly considered 3-D structures. Ever since its first discovery in the early 1990s by Sumio Iijima,[10,11] currently a senior researcher at the NEC Corporation in Tsukuba, Japan; CNTs have generated great interest among nanotechnology researchers worldwide, and they have been hailed as the most promising candidate for the development of nanoelectronics. CNTs, especially single-

Figure 3a: Physical structure of a N-type transistor. **Figure 3b:** The i_D–v_{GS}, drain current vs. gate voltage, characteristic for an N-type transistor in saturation (V_t= 1V). (Source: Sedra and Smith.[6])



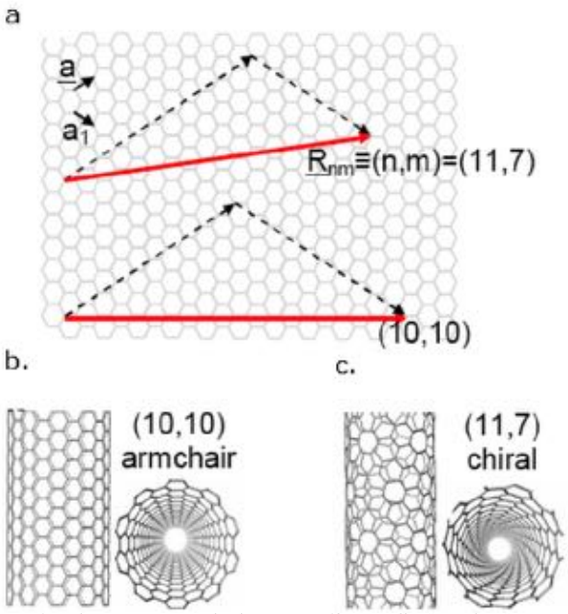


Figure 4a: The wrapping methods. **Figure 4b:** Metallic "armchair" structure. **Figure 4c:** Semiconducting "chiral" structure. (Source: Javey and Dai.[1])

walled nanotubes, possess unique electrical properties that may enable the delivery of superb performance and extremely low power consumption. Moreover, carbon nanotubes have been used to produce several prototype devices (e.g., novel top-gated transistor prototype[12]) in laboratories worldwide. These devices were observed to deliver better performance in some technical aspects, such as transconductance and current density, than current silicon technology.[12,13,14,19,21]

Structure of Single-Walled Carbon Nanotubes

As previously mentioned, carbon nanotubes are two-dimensional (2-D) graphene sheets rolled up to become essentially 1-D tubes. It has been theoretically and experimentally shown that the electrical properties of the SWNTs can be altered by wrapping up the graphene sheets in different ways. The various "wrapping methods" and the resulting types of SWNTs are generally described using a sheet of honeycomb structured graphene, presented in Fig. 4.

The two vectors **a** and $\mathbf{a}_1$ in the hexagon (Fig. 4a) are known as unit vectors of graphene in real space, and the pair of integers (n,m) indicates the number of unit vectors along two directions of the hexagonal lattice, and the chiral vector, along which the graphene sheet is rolled up, is the vector sum of the two unit vectors multiplied by the indices, or mathematically

$$\mathbf{R}_{nm} = n\mathbf{a}_1 + m\mathbf{a}_2 \qquad (2)$$

If $n = m$, the resulting nanotube is known as the "armchair" structure (Fig. 4b), which is experimentally equivalent to a metal. When $n - m \neq 3j$, the nanotube is semiconducting, and known as the "chiral" structure (Fig. 4c).[1,13] *McEuen* et al.[12] state that in an SWNT, the momentum of the electrons moving around the circumference of the nanotube is quantized, and such quantization results in tubes that are either one-dimensional metals or semiconductors.

Synthesis of Carbon Nanotubes

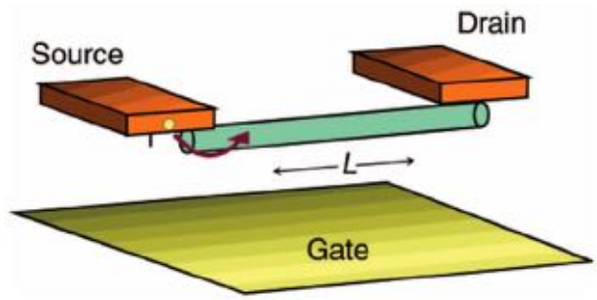
Several synthesis methods have already been developed to date, though virtually none of them are mature enough to be cost-efficiently exploited for mass commercial purpose. The synthesis method introduced here, known as catalyst chemical vapor deposition (CCVD), is a gaseous carbon source-based synthesis that is relatively more efficient than other currently available methods (such as laser ablation[4]). The CCVD allows the nanotube growth to directly take place on a silicon wafer that has catalyst material such as iron placed on its surface. The wafer is then exposed to a flow of carbon source gas, such as methane (CH_4), in a standard furnace, which provides heating at 660°C ~ 1000 °C. The carbon atoms from the decomposed methane gas can then condense on the cooler substrate, resulting in the growth of carbon nanotubes from the catalyst seeds that were previously placed on the substrate. The CCVD yields larger quantities of carbon nanotubes and offers more flexible control over the properties of the nanotubes in terms of engineering the properties of the catalyst and adjusting the growth conditions.[12] Therefore the CCVD method is more favorable to large scale manufacturing of carbon nanotubes. Yet, some technical difficulties (e.g. growth of uniform CNTs) need to be solved before CCVD can become a reliable, cost-effective synthesis method for commercial and industrial CNTs.

Electrical Properties of Carbon Nanotubes

Although study of the electrical properties of CNTs is still ongoing, some significant discoveries have been made and important theories have been established. The unique electrical properties are largely derived from the 1-D characteristic and the peculiar electronic structure of graphite.[19] Further two factors determine the conductivity (metallic or semiconducting) of a CNT—chirality (ways of wrapping) and the diameter of the tube.

Generally, the conductance of a carbon nanotube is measured by attaching each end of the tube to an electrode, and varying the gate voltage (V_g) applied to the nanotube from a third terminal. If the conductance is relatively independent of V_g, the tube is considered metallic; if apparent variation of the conductance in response to V_g is observed, then the tube is semiconducting. The conductance G of a carbon nanotube is given by equation (2). *McEuen* et al.[12] point out that $N = 4$ for a SWNT at low doping levels such that only one transverse sub-band is occupied, and thus the conductance of a ballistic SWNT with perfect contacts ($T = 1$) between the tube and the electrodes is $4e^2/h = 155$ μS. This gives a corresponding resistance of 6.5 kΩ, which is the unavoidable fundamental contact resistance. Imperfect contacts additionally cause a resistance $\mathbf{R}_c$, while the presence of scatters (e.g. defects in the nanotube) that give a mean free path le for backscattering (electrons colliding with defect and consequently bounce

Figure 5: Measuring the conductance of a carbon nanotube. (Source: P. McEuen and J. Park.[14])



backwards) contributes an ohmic resistance denoted as

$$\mathbf{R}_t = (h/4e^2)L/\boldsymbol{l}_e \qquad (3)$$

where L is the length of the nanotube.[12] Therefore the total resistance is given by the sum of the three:[12,14]

$$\mathbf{R} = h/4e^2+\mathbf{R}_c+\mathbf{R}_t \qquad (4)$$

In semiconducting nanotubes, the diameter of the tube affects conductivity through the bandgap, because of quantum mechanical effects. The bandgap of semiconducting CNTs is given by

$$E_g = 0.9\text{eV}/d \qquad (5)$$

where d is the diameter of the tube in nanometers.[14] This indicates that the bandgap can be changed by controlling the diameter of the nanotube. The larger the tube diameter, the smaller the bandgap. Compared to those of silicon and gallium arsenide (GaAs), which have values of 1.12 eV and 1.42 eV respectively, a semiconducting CNT has a smaller bandgap and hence requires less external energy, such as thermal excitation, to be made conducting. By increasing the diameter, the bandgap energy of CNT can be reduced to a level comparable to that of germanium, which is 0.66 eV.[3]

Besides their excellent feature of controllable conductivity, there are several other electrical properties that make CNTs attractive. For example, CNTs have extremely low electrical resistance. Resistance stems from collisions between electrons and defects in the crystal structure of the material in which the electrons are traveling. However, for electrons traveling in the nanotubes, the 1-D structure of the CNT limits propagation path to one-directional , eliminating any other angles for electron scattering (such as occurs in 3-D conductors), and thus greatly increasing the efficiency of electron transport. Electrical resistance in CNTs may occur under the circumstance of backscattering, in which the direction of electron motion changes abruptly from forward to backward. However, backscattering requires strong collisions under higher voltage bias[12,19] and hence is less likely to happen. In addition, the ballistic electron transport range is fairly long compared to high-quality compound semiconductors (e.g. GaAs)—a few micrometers in metallic CNTs and several hundred nanometers in semiconducting ones.[5,19] Low resistance enables faster current flow (thus faster operating speeds) and lower power consumption. In metal wires, the resistance is inversely proportional to the cross-section area of the wire, leading to increasing power consumption with device down-scaling. Heat generated as a result of high power consumption can also possibly melt the metal wires. On the other hand, in addition to low resistance, CNTs (metallic) are capable of withstanding extremely high current densities (up to ~ 10^9A/cm^2, > 1000 times greater than metals like copper and silver)[13] and conducting heat very well,[4] making them perfect candidates for interconnects and heat sinks in electronic circuits.

The last remarkable electrical property to be discussed is the surface state of CNTs. The type of chemical bonds that atoms can make varies, depending on the chemical element. In the case of silicon (Si), the material widely used in modern electronics, each atom in the interior crystal bonds with four other nearby atoms. However, on the surface, there exist atoms that are not fully bonded. These atoms can trap wandering electrons, resulting in unwanted charged sites that may eventually degrade the device function. To solve this problem, one current technique is to expose the silicon surface to oxygen, which bonds with the silicon atoms to form an oxide thin film. However, the carbon atoms in nanotubes don't have any unbonded atoms, and thus eliminate the need to grow a thin film on the surface or limit the options of gate insulator to only silicon oxide. This is significant because other superior materials can be chosen that help minimize the possibility of electrons tunneling into the gate, which can consequently degrade the device performance.[19]

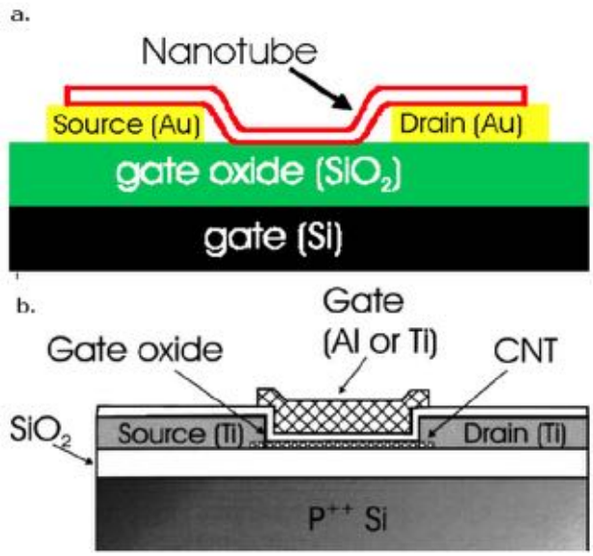


Figure 6a: Back–gated CNT-FET prototype. **Figure 6b:** Top–gated CNT-FET prototype. (Source: (a) IBM[15] (b) *S.J. Wind* et al.[16])

Technological Applications of Carbon Nanotubes

Given their unique electrical properties, robust mechanical strength, and excellent thermal conductivity, CNTs have wide applications in current and future technologies. In 2002, researchers demonstrated the top-gated, CNT-based FET (Fig. 6b) that offered more flexible control on the operation of individual devices compared to an earlier, conventional back-gated transistor prototype (Fig. 6a).[16] This device has further shown to have significantly better transconductance (the measure of the change in channel current with respect to gate voltage change) than current-generation MOSFETs. Combined with other technical aspects of the device, such as turn-on voltage and I_{on}/I_{off} ratio, the researchers concluded that performance potential of the CNT-FETs may rival, if not exceed, that of state-of-the-art silicon-based MOSFETs.[12,17,18]

Besides their use as devices, carbon nanotubes have other applications. A few typical, electronics-related applications are listed below:

Field Emission: Field Emission can be described as the emission of electrons from the ends of a nanotube when a small electric field is applied parallel to its axis.[4] An attractive application of this effect of the carbon nanotubes is flat-panel displays. Motorola reported that the method they developed can be used to produce a 50 inch display that is superior in brightness and power consumption and cheaper than other types of flat-panel displays such as LCD and plasma.[25]

Chemical sensors (CNT transistor-based): Although the high-performance processors that demand uniform, high-quality carbon nanotubes won't be available soon, CNT-FETs can be used to produce highly sensitive chemical sensors that can work with a mix of different nanotubes. Because of their high

sensitivity of conductance to local chemical environment, CNT-FETs are unique as detectors of various chemical gases.[22]

Technical Challenges in Carbon Nanotubes

Several technical challenges must be addressed before carbon nanotubes can become a viable technology for the electronic industry. These challenges, to a large extent, stem from manufacturing issues. Due to the extremely close connection between the geometric structure of CNTs and their properties (electrical properties in particular), a much more detailed, mature understanding of CNT growth mechanism is highly desired. Current challenges in carbon nanotubes (for use in electronics) include lack of precise control of the synthesis process to produce CNTs with desired diameter and chirality,[24] high synthesis temperature (incompatible with many other standard silicon processes),[14] inefficient method of sorting of bundles of nanotubes, immature ability of precise positioning of nanotubes on silicon wafer, and high resistance contact between source/drain electrodes and the CNTs. Current synthesis methods for producing CNTs in larger quantities often result in producing a mix of metallic and semiconducting CNTs that tend to bundle up together. It is necessary to be able to efficiently separate CNTs of different metallicities as well as keep them from sticking together. The problem of precisely positioning large number of nanotubes in between corresponding source/drain electrodes on the silicon wafer also needs to be solved. Lower contact resistance between the metal electrodes and the CNTs is desired as well (researchers are currently proposing using metallic CNTs as source/drain electrodes). Nonetheless, significant improvements have been made world-wide, for example, a novel vertical CNT growth method has been developed at Purdue University;[27] a device produced at Rice University sorts CNTs by size and conductivity;[28] a method developed at IBM T.J Watson Research Center can accurately position a certain number of CNTs;[23] a lower temperature (350°C) growth technique for single-walled nanotubes has been developed at the University of Cambridge, England.[29] The efforts devoted to tackling these challenges are succeeding in improving the field dramatically.

Nanowires (NWs)

Nanowires are another novel class of nanostructures with excellent prospects for future nanoelectronics development. They can be constructed from a variety of materials, used primarily to determine their electrical conductivity. Thus, nanowires can be classified based on the material of which they are made; for example, metallic (Au, Ni, Pt, etc.), semiconducting (Si, GaN, InP, etc.), and insulating (SiO_2, TiO_2, etc.). Although the diameter of nanowires, like that of carbon nanotubes, is one of the factors that determine their electrical transport property, this "conductivity by material" characteristic of nanowires is certainly an advantage over the carbon nanotubes, whose conductivity is determined by their chirality (recall that chirality demands more precise control during synthesis). This advantage becomes more significant especially when using nanowires as transistors, owing to the manufacturing convenience.[30]

Physical Structure of Nanowires

Nanowires, in many cases, are considered one-dimensional (1-D) nanostructures just like carbon nanotubes; although, their length-to-diameter ratio (sometimes called aspect ratio) may not be as large as that of CNTs because of their larger diameter. The diameter of nanowires could range from 10 nm to 70nm, and their lengths could be at micron to centimeter scales. This gives nanowires typical length-to-diameter ratios of 1000 or larger. Unlike SWNTs, nanowires in most cases do not have hollow interior space, and their surfaces are also not structured like the carbon crystal lattice of graphene and therefore may cause surface scattering of electrons. Nanowires made of semiconducting materials can also be doped, i.e. their electrical properties may be altered by adding impurities to their chemical structure.[30]

Electrical Transport Properties of Nanowires

As the size of a nanowire becomes sufficiently small, quantum confinement effects play a significant role in electrical transport phenomena. As another class of 1-D nanostructure, nanowires are capable of exhibiting ballistic electron transport as their lengths L are reduced to equal or smaller than the electron mean free path $\boldsymbol{l}_e$,[30,32] and electrical conductance of nanowires with N conduction channels is defined by equation (2):

$$G = N(2e^2/h)\,T$$

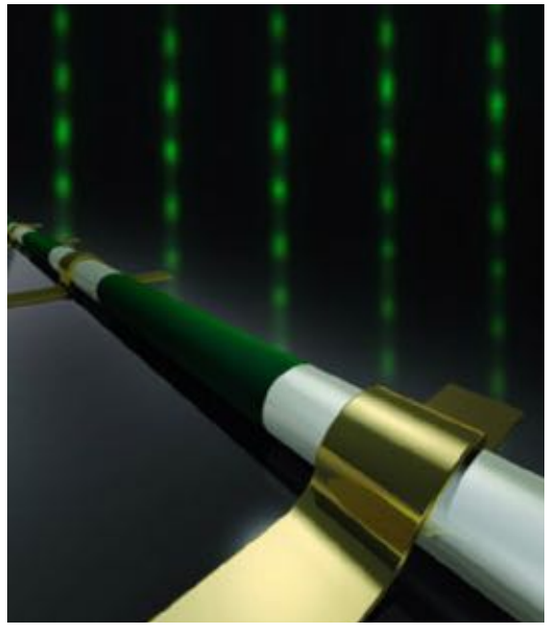
On the other hand, when a nanowire has a length greater than the electron mean free path, its electrical transport is diffusive.

Technological Applications of Nanowires

Nanowires have a wide range of potential applications in electronics, optoelectronics and (biochemical) sensor systems, owing to their small structure and unique properties. A small list of nanowire applications is presented below:

Electronics: In nanowire-based FETs, nanowires are typically deposited on an insulating substrate (e.g. SiO_2) and connected to the source and drain, then either a bottom or top gate configuration is added as the third terminal electrode to form the FET. Although it remains unclear whether NW-FETs can outperform the current state-of-the-art MOSFETs, several techniques have been developed, such as thermal annealing to improve source/drain-to-nanowire contacts and the novel

Figure 7: NiSi/Si nanowire heterostructures devices (Source: Lieber Research Group, Harvard University[31])



Germanium core/silicon shell nanowire heterostructures, and the NW-FETs exploiting these techniques have been shown to have improved performance in some technical aspects (i.e. transconductance, on-current, and carrier mobility) that are three to four times better than their current silicon MOSFET counterparts.[33] Nanowires can also be used to produce wire arrays that serve as interconnect in the circuitry.[34,35,37]

Optoelectronic: It has been demonstrated that nanowires can be predictably synthesized as n- or p-type. They can be assembled into cross-wire p-n junctions, which emit light strongly and have been said to be the smallest light-emitting diodes (LEDs) ever made. Such LEDs are potential candidates for optoelectronic applications.[36]

Biochemical sensing system: Through chemical modification on nanowire-based sensors, a group of receptors that are sensitive to specific biochemical matters are linked to the surface of nanowire connected between the source and drain electrodes on a p-type NW-FET. Upon the binding event between the receptors and the biochemical molecules (e.g. protein), the increased positive charges on the NW surface decrease the conductance of the p-type NW-FET. This serves as an electrical signal to be processed.[38,39]

Limitations and Challenges in Nanowires:

The conductivity of nanowires is expected to be less than that of carbon nanotubes. This problem largely arises from nanowires' surface condition. Unlike carbon nanotubes, nanowires are made from a variety of materials, and their surfaces may have unbonded atoms. These atoms can potentially become sites of defects that trap excessive charges, which negatively affect the conductance of the nanowire. Such edge effects can grow more severe as nanowires shrink in size and their surface-to-volume ratios become larger. Moreover, as discussed, ballistic electrical transport can occur only when the wire length is reduced to the scale of the carrier mean free path. Nanowires that do not meet this requirement will experience numerous electron scattering events.

Some other challenges include manufacturing cost and integration difficulty. Precise control over nanowire growth has been achieved in laboratory. However, according to Lieber, currently the cost of developing large-scale manufacturing would probably not be justified by a 4 to 5 times improvement in performance over current technology.[42] For the integration into nanosystem circuitry using nanowire arrays as interconnects or building blocks, the challenge lies in finding effective processes for building contact structures. Researchers at the California Nanosystems Institute claimed that wire arrays they created are far smaller than the resolution obtainable with electron-beam lithography, so integrating them into semiconductor processes will be difficult.[40]

Quantum Dots

Quantum dots, also known as semiconductor nanocrystals, are a special class of semiconductor nanostructure. Since they are capable of emitting electromagnetic radiation (especially visible light) when given external stimulus, quantum dots are also known as fluorescent semiconductor nanoparticles. Their unique optical tunability and photovoltaic characteristics enable a wide range of current and potential applications in optoelectronics and other fields such as life science.

Structure of Quantum Dots

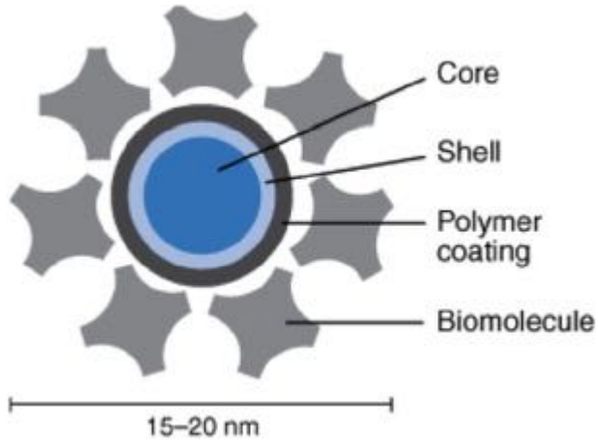


Figure 8: Structure of a quantum dot produced at Quantum Dot Corporation (Source: Quantum Dot Corporation[44])

Quantum dots have typical diameters ranging from 2 to 10 nanometers. The overall structure of a quantum dot consists of a core, a shell, an outer coating, and may have bio-molecules attached to the surface of the coating. The core of the QD is enclosed within the shell, whose outer surface is entirely occupied by the polymer coating, with bio-molecules as external attachments.[44] QDs produced at Evident Technologies have structures that consist of only cores and shells.

Properties of Quantum Dots

The uniqueness and usefulness of QDs stem from their tunable optoelectronic properties. The fluorescent nature of QDs enables them to absorb external stimulus (e.g. thermal excitation, voltage, and laser flux) and re-emit light of various wavelengths. As mentioned, when given enough energy, a number of electrons can jump across the bandgap into the conduction band momentarily. While falling across the bandgap back to the valence band, depending on the size of the bandgap and the energy they lose during the transition, the electrons emit electromagnetic radiations of different wavelengths. The emission wavelength is fixed in bulk semiconductor materials because of their fixed bandgap. On the other hand, the tunability of the QDs is made possible through the precise control of QDs' sizes, which in turn determine the size of the bandgap and peak emission frequency of the electrons. As a QD becomes sufficiently small, the size of its semiconductor crystal decreases to equal or smaller than the Exciton Bohr Radius, known as the separation distance between the electron and the hole in single exciton pair,[45] and consequently the energy levels become more discrete and the bandgap increases. As a result, the energy electrons lose when falling across the bandgap becomes larger, and the emitted wavelengths become shorter, leading to "blue-shifted" light (more towards the blue end of spectrum). The light emitted, therefore, appears to be redder for QDs of larger sizes.[43]

Applications of Quantum Dots

The tunable optical property grants QDs a variety of optoelectronic applications, such as light-emitting diodes (LEDs) and quantum dot lasers. Researchers at Vanderbilt University, for example, have discovered that CdSe quantum dots are able to emit white light when excited with an ultraviolet laser. Moreover, QDs can also be used in solar cells. In 2004, scientists at Los Alamos National Laboratory demonstrated

that impact ionization in PbSe quantum dots could occur at very high efficiency, which may lead to considerable increase in power conservation efficiency of QD-based solar cells.[49,50,51,52]

Challenges

The challenge to date for QDs is the cost of manufacture, with the majority of it originating from the synthesis solvent used to produce quantum dots. One gram of CdSe QDs produced at Evident Technologies Inc. cost $6399 (as of January 2005), while the same quantity of CdSe QDs made at NN-Labs Inc. cost $2100-$3375 (as of March 2005). The research team led by Professor Michael Wong at Rice University have recently reported a method of using heat transfer fluids as synthesis solvent instead of conventional octadecene can help cut the cost down by 80%. The next step according to him is to develop a continuous-flow system for making quantum dots in kilogram amounts.[46,47]

Summary

In this paper, the structure, properties, applications, and technical challenges of carbon nanotubes, nanowires, and quantum dots were discussed in detail. In addition, the synthesis of carbon nanotubes was also covered. Since quantum effects play a predominant role in the properties of these nanostructures, the energy band model was presented to help the reader understand fundamental concepts. Since the electron transport properties in larger materials differ significantly from those of nanostructures, concepts of ballistic electron transport and conductance of one-dimensional nanostructures (i.e. carbon nanotubes and nanowires) were also discussed in detail. On the other hand, the unique electrical properties of quantum dots as three-dimensional nanostructures reside in their tunability, enabling them to be promising candidates for optoelectronics applications. However, by observing the technological challenges, one can reasonably claim that research and development of nanotechnology applications in electronics are still at the early stage, and nanotechnology is not very likely to replace the current semiconductor technology as a predominant force in electronics industry soon. Owing to decades of development, semiconductor technology has the maturity that current nanotechnology does not have. Nonetheless, superior properties of novel nanostructures have been shown. While the challenges are still being overcome, one can take advantage of the superiorities of these nanostructures and use them as "components", such as building circuit interconnects with CNTs or nanowires, which are incorporated into semiconductor technology to extend its physical limit. Moreover, the advantages of nanotechnology and other competing semiconductor-based technologies can also be possibly combined to create better devices. As design, testing, and manufacturing techniques of nanotechnology gradually mature, it can undoubtedly become a leading force that has ubiquitous influence in the electronics industry.

Acknowledgement

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truth® or Consequences: An analysis of public service announcements in contemporary society

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The pervasiveness of advertising is continuously increasing in today's society. Though there is no agreed-upon statistic, the most frequently cited estimate proposes that Americans encounter 3000 ads every day.[1] In most industries, advertising plays a central role in the development of successful businesses. But advertising's influence extends far beyond that of a simple economic supplier. The advertising industry is so powerful and so essential to companies today that business decisions and media content are dictated and limited by the fear of losing advertisers' backing. Controversial television shows and news stories are thus weighed against the potential loss of advertising revenue. Moreover, advertising has become so prevalent that newspapers now allocate 50 to 65 percent of their space to ad content so that it is the ads and not the news which are positioned first in the newspaper. Only then is the remaining space – the "newshole" – filled with the actual news content.[2]

The omnipresence of contemporary advertising has created a society that is made up not of citizens but of consumers. Individuals are bombarded and consequently molded by not-so-subtle messages that products, not personalities or character traits, make people "cool" or sexually attractive. The tobacco industry in particular has perfected this art, especially in its marketing toward teens, promoting its products by glamorizing smoking for nearly a century, aligning cigarettes with images of the cool and the rebellious. However, cigarette promotion is far different from any other product campaign. As an addictive and deadly product, the implications of cigarette advertising cannot be equated with ads for lipstick or soda.

Unfortunately, the success of the tobacco industry and its extensive marketing campaigns has placed cigarette ads in a category of their own. Classic tactics include, as mentioned above, emphasizing cigarettes' ability to make a person appear cool, sexy, and more masculine or feminine. Through the use of celebrity endorsements in the 1950s, the Marlboro Man's ruggedness, and the more modern tradition of attractive, nearly-naked models promoting smoking, the tobacco industry has been successful in glamorizing smoking and establishing a positive image for its product. These notions have not merely been introduced to society; rather, they have become inescapably ingrained in the contemporary mindset.

Yet with the growing awareness of nicotine's fatal qualities, more and more antismoking campaigns are being developed today to counter the tobacco industry-established belief that cigarettes breed sophistication. These types of campaigns face numerous obstacles in not only reaching people with their message, but actively instilling the message in the mind of the public. The problem that arises with social awareness advertising boils down to one fact: selling a message is a great deal more difficult and complex than selling a tangible product. Specifically with antismoking messages, campaign organizers must additionally combat the billions of dollars allocated by the tobacco industry for advertisements. "According to the Federal Trade Commission, tobacco companies spent more than $15.1 billion in 2003 to market their product in the United States, up from $12.4 billion in 2002."[3] Moreover, Colleen Stevens, chief of the tobacco media unit at the California Department of Health Services notes, "When something is as much a part of your culture as smoking, there is no one [message] that stops it."[4]

These dilemmas raise questions of how to target a nation that has been incessantly bombarded by advertisements, so much so that individuals have become molded by advertisers into the perfect submissive consumers – consumers who have been programmed to buy what they are told will make them happy and take up habits which they are deceptively taught to think are cool. Unfortunately, a policy has materialized where consumers no longer interrogate what is placed before them, leading to a society that is not only consumer-driven, but unquestioning of advertisers' manipulations. The American Legacy Foundation's antismoking campaign – truth® – fights to rattle the submissiveness of citizens and present a no-holds-barred view of the tobacco industry. In what has been widely deemed a "hard-hitting" series of campaigns, truth® turns advertising conventions around, using new methods (especially as compared to traditional public service announcements) to produce a unique message that has successfully lowered smoking rates among teens. However, before examining the creative tactics of the truth® campaign, it is necessary to first explore the tobacco industry and its ad campaigns – specifically focusing on the iconic image of the Marlboro Man – and to contextualize public service announcements through a discussion of early anti-drug ads.

The Tobacco Industry and its Glamorization of Smoking

The advertising methods of the tobacco industry are fairly limited given the harmful nature of its products. Since advertisers have no concrete benefits to tout, they have consequently mastered the technique of associating cigarettes with positive images and objects. Earlier ads relied on celebrities to endorse and thus glamorize smoking, exploiting the public's continued fascination with stardom. Other tactics included the portrayal of upper-class smokers promoting various brands of cigarettes. In a Camels ad from 1934, a well-dressed young woman is presented in a seemingly plush and luxurious environment. The ad describes "Why Miss Anne Gould, daughter of Mr. and Mrs. Jay Gould, prefers Camels."[5] The full title of her name and the formal address, along with the background setting, indicate that Miss Anne Gould is a representative of a higher class and its choice of cigarettes. This particular ad equates Camels with a glamorous and attractive lifestyle which the common man or woman can mimic by smoking Camel cigarettes.

More contemporary advertising has moved away from these suggestions of sophistication. Glamour has been replaced with a similar but more modern concept – the concept of cool. The idea of "cool" embodies so much, but essentially it represents mainstream (particularly teenage) aspirations to fit in, be liked, and at best, be considered popular. Advertisers take advantage of these insecurities and desires, offering their products as a means to achieve such status. This occurs on even on the most basic level. Take for instance the brand of cigarettes named "Kool." How much more blatantly could a manufacturer send the message that a cigarette can provide social acceptance?

Figure 1: Camel ad, 1934.[6]

A step beyond this is the concept of gender definition, a more specific form of the notion of cool. Associations between a product and enhanced femininity or masculinity play off desires to fit into the respective genders. In terms of cigarette advertising, different brands have been specifically geared to either males or females and have thus been marketed on this basis. To fully understand the use of gender in cigarette ads it is helpful to turn to what Advertising Age designated the number one icon of the 20th century – the Marlboro Man.[7]

The Marlboro Man's Iconic Status

Introduced by Philip Morris in the mid-1950s, the Marlboro Man was actually developed as a marketing strategy designed to boost sales by abandoning Marlboro's image as a female-oriented brand. The slogan "Mild as May" was replaced by a rugged cowboy oozing with masculinity. The Marlboro Man is presented as the epitome of what it means to be "A Man." Moreover, the sexual implications carried by the Marlboro Man's masculinity appeal both to women who want him and men who desire to be like him (and be equally attractive to women). The success of the Marlboro icon completely embodies the advertising tactics used by the tobacco industry. Through strategic framing and imagery, the Marlboro Man represents all of the positive implications that Philip Morris wishes to associate with smoking. Furthermore, the coolness and machismo personified by the Marlboro Man have withstood with test of time and remain effective even today.

The image of the cowboy has long been attached to notions of individualism, masculinity, strength, and ruggedness; the Philip Morris campaign took this a step further by emphasizing the sexuality of these figures. Take, for instance, the two ads on the following page.

The stance of the men in each is the same; moreover, the pose is one that just so happens to focus on the crotch area. The leftmost ad more strongly highlights the area through the careful placement of the "Marlboro" label just above the crotch, the position of the cowboy's left hand, and the contrast between his pants and his chaps. Further deconstruction of the ad proves that the careful positioning of each element in the ad is meant to ultimately lead the viewer to the crotch – the representation of the Marlboro Man's masculinity as well as his sexual appeal. The bright red Marlboro name stands out against the muted denim jacket and is one of the first elements viewers notice. The end of the word "Marlboro" leads viewers to the cowboy's left elbow, which in turn leads down to his hand and directly to the crotch area. Additionally, the sunlit chap on the left mimics the color of the man's hand on the right and creates an arrow toward the target spot of the crotch.

The second ad, though not as overt, still emphasizes the sexual components of the imagery. Here sexual innuendo is used to highlight the Marlboro Man's sexuality. The slogan – "Come to where the flavor is" – is one that has been used in many Marlboro ads; however, given the particular placement in the above ad, it can be read here as having sexual implications. By situating the phrase directly above the cowboy's bent knee, the ad is insinuating that the flavor is located in the crotch of the Marlboro Man. Viewers are not only being enticed to pick up a Marlboro cigarette because of the brand's individual flavor but are being enticed by the Marlboro Man's overt sexuality.

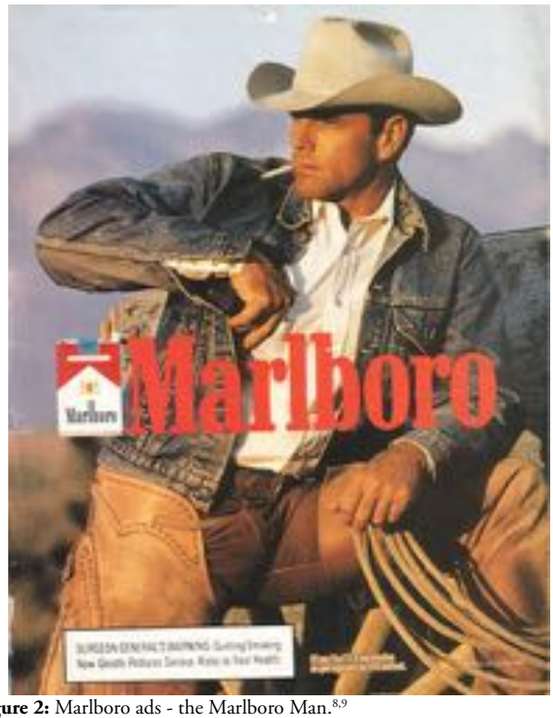

Figure 2: Marlboro ads - the Marlboro Man.[8,9]

The example of the Marlboro Man is indicative of the methods the tobacco industry has used to ingrain certain notions within society's mind – that smoking is cool and/or glamorous, that it strengthens one's masculinity or femininity, that it is a mark of sexuality and thus increases one's sexual desirability. Moreover, the iconic status of the Marlboro Man is apparent in the following anecdote. A fifth-grader submitted an entry to an antismoking-poster contest held by a New York nonprofit in the early 1990s. The child's piece portrays "a skeleton with a cowboy hat, riding a horse into a cemetery" and includes the statement "Come to where the cancer is."[10] This direct parody of the Marlboro campaign and its slogan "Come to where the flavor is" exemplifies the pervasive and identifiable nature of the Marlboro brand. More significantly, the ability of a child – one with only eleven years' worth of exposure to advertisements – to reconstruct the meaning of the symbolic Marlboro Man speaks volumes about the image's recognizable status in contemporary society. While the message of the fifth-grader's poster is ultimately a positive declaration against smoking, the fact that such a young adolescent could produce this level of parody illustrates the continuing need for antismoking campaigns. Ultimately, however, the power of the Marlboro icon proves the difficulty of the antismoking campaigns' mission, and creators of public service announcements are left to battle the longstanding and compelling idea that smoking is glamorous.

Attempting to Change Public Sentiments

The main purpose of the public service announcement (PSA), a type of noncommercial advertisement, is to educate the public. According to the Ad Council, a leading creator of PSAs in the United States, the most important aspect of awareness advertising is to "mobilize" the public and "make lasting and positive social change."[11] Cigarette and drug usage are key topics of public service campaigns given their known health risks and connection to adolescents. Unfortunately, teens are a particularly hard demographic to reach with important social messages and PSA creators have long struggled to produce effective ads that actually influence teenagers to change their perceptions of cigarettes and drugs.

This manner of advertising was initially introduced in the 1980s as a result of First Lady Nancy Reagan's push to bring the "War on Drugs" to the forefront of American public issues. Her effort to educate U.S. society – particularly teens – about the dangers of drug use and abuse gave rise to a television campaign bearing the title "Just Say No." An attempt to empower and enlighten kids regarding their ability to become active agents against peer-pressured drug use, the campaign challenged teens to follow the campaign's title and "Just Say No." One campaign commercial shows an array of adolescents – boys, girls, whites, blacks – all being solicited to try various drugs by an off-screen husky male voice. Each child continuously rejects the man's offer with a resounding and confident "No," apparently somewhat disgusted and almost taken aback by the assumption that they may be interested in his illegal proposition. A male teen provides a voiceover, which is interspersed with the queries of the older man and the rejections of the teens. "If someone offers you drugs, instead of saying something you really don't mean, just say 'No!'" He continues: "No big production number, just say 'No!' you'd be surprised at how well it works." In both instances, the statements of "No!" are voiced diegetically by the adolescents.[12]

The exaggerated portrayal of the manipulation and pressure that teens face in regard to drug temptation is an attempt to elicit fear and anxiety, for teens themselves and also for parents. But beyond parents' concerns about abduction and forced drug use, the lurking, unseen man represents the ever-present apprehension teens experience daily in regard to peer pressure. The commercial thus endeavors to allay these fears by offering adolescents an alternative method to the influence of peer

pressure. The simple answer to kids' problems – "Just Say No" – is meant to be the obvious solution that was missing from public discourse for so many years.

Unfortunately however, this commercial fails on many levels. Though the time period in which it was aired must be taken into consideration – public conversation about drug use was not as open and realistic during the 1980s – the entire ad campaign lacks numerous components that prevent it from effectively instilling anti-drug sentiments in America's youth. First and foremost, the slogan and premise of the entire campaign, "Just Say No," offers no motivation or reasoning to support its assertion. Why shouldn't individuals use drugs? What is bad about drugs? Essentially, why should a teen say no? There is no information given citing the dangers of drugs or the possible long-term effects. The implication of the campaign is clear – drugs are bad, don't do them – but the lack of concrete proof gives teen viewers absolutely no reason to buy into the ad's message and reject drugs in the future.

Moreover, it might even be argued that the furtive role of the off-screen narrator could potentially intrigue teens about the mysterious (and consequently alluring) life of a drug user. Though his hushed, barely audible voice is clearly meant to minimize the impact of the drug names presented in the commercial, it is possible that some teens might perceive him as a tempting figure of clandestine authority. Additionally, when adolescents are explicitly told what to do they often consciously choose to do the opposite. While the campaign attempts to combat this by offering, but not forcing, a clear-cut solution, a firmly rebellious child might reject such an unrealistic and oversimplified line.

As a result of the ineffectively argued stance of the "Just Say No" campaign, as well as the lack of reasoning behind its slogan, later PSAs attempted to portray more assertive anti-drug messages. In 1998, the Partnership for a Drug-Free America produced a commercial which veered away from the non-aggressive tone of the "Just Say No" ads. Playing off an earlier commercial that the organization had created in 1987 – which showed an egg frying in a pan to represent "your brain on drugs" – the later ad took the original one step further.

The 1998 ad does not just present an egg and a frying pan as a metaphor for the damage drugs cause human brains; it recontextualizes the overall harm that stems from drug use. A young woman holds up an egg and, mimicking the 1987 commercial, states: "This is your brain." She then gestures with the frying pan in her other hand. "This is heroin."[13] Looking directly at the camera she announces "This is what happens to your brain after snorting heroin" and then proceeds to violently smash the egg with the frying pan. "And this," she declares as egg yolk drips down her arm, "is what your body goes through." "It's not over yet," she continues. "This is what your friends go through. And your family. And your money. And your job. And your self-respect. And your future." Her monologue is interspersed between shots of the passionate and aggressive anger she takes out on the kitchen. She completely demolishes the contents of the entire room, breaking and shattering dishes, glasses, a lamp, even the clock on the wall. Finally, a black screen takes over, displaying the commercial's sponsors in white lettering: "Office of National Drug Control Policy; Partnership for a Drug-Free America." But the woman is allowed the last word, and the now-wrecked kitchen reappears. Tossing the frying pan aside and squarely looking at the camera she inquires, "Any questions?"[14]

The aesthetic and cinematic components of the commercial emphasize the more assertive nature of this ad as compared to both the 1987 version and the entire "Just Say No" campaign. The color palette is made up of muted grays and whites, which add to the dimness, despair, and grittiness of the entire scenario. Moreover, the commercial represents a fast-paced and thus teen-directed approach, utilizing tools that will specifically catch the eye of the adolescent viewer the ad is targeting. Lastly, the commercial employs unconventional methods of cinematography such as jump cuts and awkward angles. These tools allow the commercial to stand apart from mainstream advertisements, but more, so they emphasize the jarring content and attitude of the ad.

In terms of anti-drug strategies, this commercial's attempt to elicit fear is considerably more effective than the previous "Just Say No" commercial. The intensity of the woman's actions is both unforgettable and haunting. Moreover, the extremity of those actions leaves viewers in a state of shock. While this commercial certainly provides more concrete reasons not to do drugs than "Just Say No," it could also be construed as telling teens what they already know – that drugs are harmful, dangerous, and detrimental. Furthermore, the ad's excessive and radical nature creates a possible situation where kids may dismiss it as unbelievable, and perhaps even as humorous.

An article written shortly after the debut of the commercial presents the reactions of numerous adolescents to the ad itself. The article outlines why one student thought the commercial was unsuccessful: "The frying pan ad failed to tell the audience what really happens when someone uses drugs and what effect it has on their family."[15] In the 15-year-old's own words, "It was kind of phony. The lady with the frying pan – that just made me laugh."[16] By indicating his acknowledgement of the ad's construction and the unrealistic situation it presents, he also reveals the dangers of creating overdramatic public service announcements. Ultimately, the article sums up a theory by psychologist Robert B. Butterworth, noting that "Evidence suggests these ad campaigns fail to deter drug use and don't connect with students beyond a mild chuckle."[17] According to Butterworth himself, "It's an MTV visual that won't make an impact."[18] This use of MTV-style imagery is indeed an attempt to specifically target a teenage audience, but as Butterworth points out, appropriate visuals don't ensure the desired response.

Butterworth's points are significant in understanding the failure of even this later attempt to reach teens with an effective and compelling message. The fact that these ads do not successfully "connect" with teens points to the root of a greater problem: to get individuals to truly accept a message and, more important, persuade them to change an ingrained belief, the sender of that message must be keyed into the mindset of the target audience. The damage drugs cause not only to the drug user but also to surrounding loved ones certainly gives one pause, but for teens this notion is easily dismissed after the initial impact wears off. Adolescents often require instant gratification and immediate reasoning. Explanations based on intangible notions regarding the consequences of drug use don't reverberate in the minds of teens. Lisa Unsworth, the executive vice president of a Boston ad agency responsible for

state government-sponsored anti-drug ads, says, "Kids think they will live forever. Talking about a disease you may get when you're 50 or 60 isn't a compelling motivator."[19] The "Your Brain on Drugs" commercial doesn't even point to such a specific outcome as a disease; rather the incentive is based solely on the intangible notion that family and friends will go through an experience equivalent to a kitchen being demolished.

Though the powerful imagery of this ad cannot be wholly dismissed, it must be understood that teens will not effectively absorb the core objective of the ad. The core objective must be distinguished from the surface-level message: drugs are bad and using them will have consequences. However, the core objective represents the deeper essence of the ad that relates to effecting tangible behavioral changes in viewers. By 1998 it was no secret that drugs were harmful, but again, teens were not fazed or disconcerted by this elusive fact. The only way that any anti-drug ad can be effective is if it can successfully turn teens into active agents against the very same outlets associated with teenage rebellion. The "Your Brain on Drugs" commercials and the "Just Say No" campaign each fail on this count and thus can only be used to demonstrate ineffective modes of public service announcements.

Changing Conventions Through the truth®

As a result of the ineffective public service campaigns of the past, new methods and different approaches to PSAs became increasingly necessary to combat the continued presence of smoking in U.S. society in the late 1990s. The growing awareness of cigarettes' deadly effects led to a major lawsuit brought against the tobacco industry ("Big Tobacco") in 1998. In what became known as the Master Settlement Agreement (MSA), 46 states[20] and five U.S. territories signed an out-of-court agreement which strove to hold Big Tobacco accountable for its alleged knowledge and concealment of the dangers of smoking and required, among other things, that tobacco companies publicly release their internal documents.[21] Additionally, the MSA led to the establishment of the American Legacy Foundation, the organization responsible for the creation of the most effective public service campaign to date – the truth® campaign.

According to its website, the American Legacy Foundation "develops national programs that address the health effects of tobacco use through grants, technical training and assistance, youth activism, strategic partnerships, counter-marketing and grass roots marketing campaigns, public relations, research and community outreach to populations disproportionately affected by the toll of tobacco."[22] More important, Legacy attempts to transform the entire societal mindset regarding smoking that has been fostered by Big Tobacco – that smoking is glamorous, cool, sexy, sophisticated, and rebellious.

The ultimate goal of the foundation provides insight into the distinct and innovative mentality of this organization: "The American Legacy Foundation is dedicated to building a world where young people reject tobacco and anyone can quit."[23] The most significant component of this objective is the first statement regarding Legacy's attempt to inspire a negative and active reaction among teens in order to motivate them to reject smoking. The intention is not just for kids to understand the well-known dangers of cigarettes, but rather, to make them rethink the concept of smoking and consciously choose to renounce tobacco products. This attitude signifies the new way of thinking, which characterizes the contemporary approach to public service announcements.

Also noteworthy is the reference to "tobacco" as opposed to "cigarettes" or "smoking." This carefully selected word choice is crucial and reflects the American Legacy Foundation's focus on the corruption of Big Tobacco as a way to discourage teens from thinking positively about smoking. This tactic of painting the tobacco industry as a manipulating and deceitful corporate entity speaks directly to the anti-authority mindset of adolescents and provides a new reason to refuse cigarettes based on moral, rather than health, reasons. In fact, "The evidence is mounting that consumer awareness about tobacco industry manipulation of consumers and of government (to obstruct tobacco regulation) is a powerful force in inducing consumers to stop using tobacco, or, in the case of youth, not to start."[24] The American Legacy foundation has thus chosen to devastate the image of Big Tobacco rather than relying on the already-known health risks of cigarettes.

The truth® campaign specifically has been described as edgy, hard-hitting, and unapologetic not only by the American Legacy Foundation itself but also by the mainstream press when describing the campaign. The Legacy website details the tactics of truth®, stating: "By telling the truth about the tobacco industry and its products and exposing their marketing tactics, truth® allows teens to make informed choices about tobacco use. It doesn't preach. It doesn't judge. It just works."[25] Though this statement might be read as biased or even self-righteous, in fact it has been statistically proven that the truth® campaign was a factor in reducing youth smoking. The results of a study published in the March 2005 issue of the American Journal of Public Health "indicate[s] that the [truth] campaign accounted for a significant portion of the recent decline in youth smoking prevalence. We found that smoking prevalence among all students declined from 25.3% to 18.0% between 1999 and 2002 and that the campaign accounted for approximately 22% of this decline."[26]

These impressive statistics demonstrate and confirm the effectiveness of the truth® advertisements and prove that it has far outstripped the past tactics of eliciting fear or portraying the immediate harm caused by drugs. The truth® ads have found a way "to change what is considered normal," getting at the root of longstanding societal conventions and altering the way those conventions are perceived by the general public.[27] Peggy Conlon, president and CEO of the Ad Council, recognizes these antismoking ads, noting that "They're making kids appear smart if they resist smoking."[28] The new-fangled notion presented by truth® – that in fact it may be wiser and even cooler to reject cigarettes – transforms the age-old "Just Say No" mentality and provides new reasoning to follow the advice of the slogan.

The website of Arnold Worldwide – the Boston-based ad agency that has paired up with Crispin Porter + Bogusky in Miami to produce the truth® campaign – outlines the mindset used in crafting the truth® advertisements. To start with, the creators differentiate this campaign from past public service announcements by allowing the facts – the truth, if you will – to speak for itself.

> *truth isn't anti-smoker or even anti-smoking. truth is pro-knowledge. truth understands that young people are smart. And*

they hate being manipulated by grown-ups. truth simply lays the facts out for teens to consider for themselves, so they can make an informed decision about smoking. Facts about the product, the industry's marketing and manufacturing practices, and ultimately the consequences. Facts that are damning enough to turn a young person's need to rebel back on the industry itself.[29]

Moreover, these two agencies demonstrate a deep understanding of their target audience, which they use to speak to teens more directly. "Think about your average teenager. They want nothing more than to demonstrate some independence and exert control over their lives."[30] Even more perceptive is the following statement regarding the reason teens smoke and what that implies for anti-smoking ads:

It turns out that young people who are open to smoking also tend to be open to other sensation-seeking behaviors. They index high on things like "daydreaming about ways to get even" or "standing up to adults" or "breaking the rules." We call them "sensation seekers". A Just-Say-No communication strategy to this group is the last thing that will work. And, unfortunately, it's the strategy that most smoking-prevention campaigns of the past have taken.[31]

This acknowledgement and analysis as to why past PSAs have failed enables Arnold and Crispin to truly grasp the necessary components of an effective anti-smoking campaign and refrain from making the same mistake as "Just Say No" or "Your Brain on Drugs."

Lastly, Arnold and Crispin recognize the rebellious, anti-authority nature of youth. At one point, rebellion came by taking up smoking; now the goal is to make choosing not to smoke seem equally rebellious. A key detail that these agencies consciously distinguish and appreciate is the fact that "if you tell teens not to do something, they'll do it."[32] Exactly the opposite of the "Just Say No" strategy, this realization engages Arnold and Crispin to make ads which will educate and appeal to teens without coming across as preachy or authoritarian:

On the rational side of the equation, truth is about honest facts and information that expose Big Tobacco or shed light on the realities and consequences of smoking. This puts teens in control. Emotionally, truth is about being rebellious, risky, intelligent, empowered, independent and tolerant.[33]

By reappropriating the rebellion once associated with cigarettes and asserting teens' ability to ultimately make their own decisions, the truth® campaign appropriately and successfully addresses its target audience.

With these insights in mind, it is now possible to examine specific examples of truth® advertisements and the way that Arnold and Crispin's recognitions translate into effective public service announcements. One commercial actually contains no spoken dialogue, but remains a particularly striking demonstration of the innovative, unique, and distinct approaches of the truth® campaign. In 2002, truth® debuted an ad (named "1200") which attempted to illustrate the number of deaths caused by cigarettes each day.[34] The spot opens as a mass of people walk through a city's streets. This mass is portrayed through a random mix of shots from various distances and camera angles – close-ups, medium-close ups, high angles, overhead shots. There are shots of faces, bodies moving forward, and the numbers on each person's chest identifying each as just one out of 1200. As the commercial progresses, more and more high-angle shots are used to depict the substantial number of people which constitute the mass gathering. Ominous music plays, mixed with clamoring noises and the sound of a helicopter to create a sense of foreboding and anticipation. As a low-angle shot pans up a glass skyscraper, a caption appears, reading "Outside a major tobacco company." The sound continues to intensify, and the camera turns to focus on the crowd below, seeming to swoop down onto the masses. Suddenly the entire crowd drops to the ground. Bodies collapse and smash against the pavement. Then, total silence. The camera intently surveys the damage – the frozen faces of the individuals, the heaps of crumpled bodies. The sound of the helicopter resumes slowly as a lone individual stands with an orange sign. It reads "Tobacco kills 1200 people a day." He flips over the sign. "Ever think about taking a day off?"

This ad doesn't use statistics alone to drive a point home; rather, it uses imagery to visualize those statistics and lend new meaning to boring and meaningless numbers. In its intensity, the commercial reestablishes the implication of 1200 people dying each day from tobacco-related illnesses. Images have long been shown to be more powerful than language, and the ad fully capitalizes on this reality. The de-emphasis of language throughout the majority of the commercial serves to bring the attention wholly to the visual elements. Moreover, the lack of words allows the viewer to interpret the ad however he or she chooses and thus prevents the message from sounding too "preachy." As Arnold and Crispin affirm, the goal is twofold – to present viewers with a set of facts and allow each individual to make of them what s/he will, but to present facts that ultimately speak for themselves.

Additionally, the commercial takes on a sarcastic tone in probing tobacco executives with the line "Ever think about taking a day off?" The ad's upfront jab at the heads of tobacco companies attracts a teenage audience in its mocking, cheeky nature and causes those viewers to engage more directly with the commercial. The reaction that this line elicits in teen viewers in turn prompts them to dissect the implication that the deadly consequences of cigarettes are unending. Moreover, "In unmasking these practices, 'truth' seeks to replace the attractive identity portrayed by tobacco advertising with a 'truth' alternative identity."[35] However, despite its ability to engage teens, the ad actually seems to be more explicitly directed at tobacco executives. Yet, the intention is not for these executives to see the ad and suddenly become aware of the ramifications of their occupation. Rather, this approach prevents teens from feeling hit over the head by a message and again refrains from any preachy sentiments. The seeming indirectness of the ad's message creates a dismissive quality which further draws a youth audience and authorizes teenage viewers to make their own meaning out of what is presented.

Another series of truth® ads titled "Crazyworld" (2003) presents an interesting view of the twisted reality of contemporary society. Highlighting the deceit of the tobacco industry even more so than the 1200 spot, this series explicitly points out the ludicrous nature of a world where Big Tobacco is permitted to promote a product it knows to be deadly. Crazyworld exposes the tobacco industry for knowing about cigarettes' dangerous effects long before this vital information was revealed to the public. The campaign largely focuses on the lack of accountability demanded of the tobacco industry, illustrated by the following paradox: cigarette packages are not required to list ingredients, yet were those same ingredients to be found in any other common consumer product, a recall

would be issued immediately.[36] The deeply ironic message that makes up the Crazyworld campaign causes viewers to stop and think much more than "Your Brain on Drugs" or "Just Say No." By imparting facts that rattle rational thought and disrupt convention, the Crazyworld ads have quite a different tone than past PSAs.

One Crazyworld ad starts by depicting a jumbled first-person point-of-view of a rollercoaster ride, symbolizing the craziness that is about to ensue. The camera proceeds to move extremely quickly and choppily, presenting a hasty montage that is both disconcerting as well as reminiscent of the contemporary MTV aesthetic. Carnival-type music plays as viewers are taken through a neighborhood setting, then the aisles of a supermarket, and a back alleyway with dumpsters. The camera is shaky. The scenes are shown upside-down, sideways, right-side-up. The camera spirals and twists through a graveyard and moves on to a fast-paced, jerky street scene. A woman's voiceover carries throughout the entire commercial:

> *Ladies and Gentlemen, hello and welcome to Crazyworld. In Crazyworld, cigarettes are the only product in the store that kills a third of those who use them. And they don't even have to list ingredients on the label. Cyanide. Arsenic. Benzene. Polonium 210. Sounds like hazardous toxic waste, right? Well in Crazyworld, those are just a few of the 69 poisons in cigarette smoke. Crazyworld is a place where light and ultra light cigarettes can be as dangerous as regular ones. And where some companies recall a product if there's the risk of harming even one customer, well tobacco companies keep on making a product that kills 1200 people a day. Crazyworld is your world folks. Take a look around. It's not funny. It's not fair. It's crazy. Welcome to Crazyworld.*[37]

The intense irony of this monologue provides viewers with a new way to look at the tobacco industry and presents anti-smoking facts in an entirely fresh light. Telling the viewer that he or she is inhaling poisons – 69 poisons – when they puff on a cigarette is considerably more effective and hard-hitting than stating that cigarettes are deadly. Actually naming the poisons is even more helpful in driving the point home. The facts become tangible through this sardonic presentation of reality versus unreality. Additionally, this approach completely reframes the discussion on smoking and successfully recontextualizes the dangers of smoking in a language that the general public can understand.

This ad and other Crazyworld examples grapple with the tension between facts so shocking and bizarre that it is hard to believe they are genuine truths. As the commercial states: "Crazyworld is your world, folks." The unsettling reality that is revealed through the equation of Crazyworld with the real world successfully compels audiences to struggle with a major question regarding what is considered "reality." The effect of leaving viewers with such a large question mark allows the message of this ad to permeate beyond the next round of commercials. Unlike "Your Brain on Drugs," which offered an initial shock but failed to follow through, Crazyworld frames facts in such a way that audiences are left unsure of the world around them and as such are asked to challenge it.

Lastly, the language and particular aesthetic tone of this commercial once again exhibit Arnold and Crispin's attentiveness to their main target audience. The sarcasm of the voiceover and the ad's overall message speak directly to teens in a manner they will both understand and respect. The buildup of the dialogue also helps to intrigue and impassion teens, instilling a more active interaction with the commercial and its message. Moreover, the visual component mimics the MTV style so ubiquitous in the adolescent world, allowing the commercial to stand out against the classical structure of most other advertisements. Not only does this jarring aesthetic quality appeal to teens, it also perfectly embodies the ad's goal of highlighting the ridiculous components of the real world. Essentially, "Crazyworld…use[s] surreal imagery and dark humor to underscore the absurdity and health risks of smoking."[38] The double impact provided by both the attitude of the message and the stylistic tone increases the effectiveness of this ad campaign.

Conclusion

The overall truth® campaign, as represented by the 1200 commercial and the Crazyworld example, has effectively pinpointed the language with which to speak to teens. Through irony, reformulated facts, and straightforward attacks on Big Tobacco, truth® has found a means to reposition the argument against smoking while maintaining a youth-friendly aesthetic and tone. By confronting the tobacco industry's dishonest and manipulative nature, truth® has stumbled upon an innovative way to frame anti-smoking sentiments through a rejection of bureaucracy and an appeal to rebellion.

Additionally, the truth® campaign creators have exhibited a strong understanding of the manner in which teens relate to facts. Tired statements to the effect of "cigarettes cause cancer" or "drugs will mess with your brain" are ineffective in that they fail to permeate to a sufficient degree. Most individuals, particularly adolescents, have difficulty thinking in the long-term and thus need statements that cannot be lightly brushed aside. Forcing people to rethink what they have never even thought to question is the only tool which causes an effective and enduring result. While the shock value is certainly a factor in several truth® ads, these ads do more than merely alarm. The campaign reframes conventional facts so that they contextualize the damage caused by the physical act of inhaling a cigarette's smoke, not the intangible notion of tobacco's harm.

Despite these key techniques so adeptly appropriated by truth®, the battle is far from over. The Marlboro Man is still one of the most recognized and lucrative advertising figures in contemporary society, so much so that "even those ad professionals who abhor the tobacco industry will, when pressed, agree that the Marlboro Man has had unprecedented success as a global marketing tool for selling Philip Morris Cos.' brand."[39] Unfortunately, the iconic status of this figure is no match for even the most effective PSAs. As an image which carries a substantial array of implications and embodies so many attractive traits, the aura of the Marlboro Man is nearly impossible to escape. Consequently, truth® is the David to Big Tobacco's Goliath in the anti-smoking battle – fighting against a strongly established industry with unparalleled resources. As stated succinctly by Arnold Worldwide: "How do we outbrand Big Tobacco with a fraction of their budget?"[40]

However, there is hope that the underdog will win this struggle. In 1929, Edward Bernays, a brilliant public relations agent, covertly commissioned a group of young New York socialites to march in the city's Easter parade each confidently smoking a cigarette and wearing a banner which read "torches of freedom."[41] Aimed to reverse the traditional notion that

the only females who smoked cigarettes were prostitutes, this publicity stunt coincided with the recent emancipation of women and was consequently presented as the women's "gesture of protest for absolute equality with men."[42] The importance of this anecdote to the current battle against smoking lies in the outcome of Bernays' gimmick. Through this wholly manufactured act, Bernays was successful in "breaking the taboo against female smoking" and causing a rise in cigarette sales.[43] This incident proves the complete construction that went into the establishment of cigarettes' status in society and gives hope to the potential ability to deconstruct this position. It took time, effort, and – as the Easter parade proves – manipulation for the tobacco industry to build to its current image, and the increasing awareness of the risks of smoking can do nothing more than dismantle Big Tobacco's precious image. Just as Bernays tapped into public attitudes to discover the best way to present cigarettes as rebellious and liberating for women, the truth® campaign is using the same tools to shed new light on the tobacco industry and reconfigure the positive perception of cigarettes in contemporary society.

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Perspectives on Research

jur interviews Assistant Professor Vera Goburnova

Vera Goburnova is an Assistant Professor of Biology at the University of Rochester

jur: Could you tell us about your educational background, how you first got

involved in research, current research interests, and outside interests?

Gorbunova: I wanted to be a scientist for as long as I can remember; it's hard to say when exactly. As a high school student, I was interested in animal behavior, which I think is a common path for biology students. We start as animal lovers, and then as we learn more, become interested in molecular questions. I did research projects at the St. Petersburg Zoo and other observations of animals, and then I decided to apply to the Biology Department at St. Petersburg State University, which is where I did my undergraduate studies; I was involved in several independent studies as an undergraduate. I would highly recommend that undergraduates go out there and find a laboratory that sounds interesting to you and get involved in research. For me, undergraduate research experience was extremely useful. It broadened my horizons and gave me first-hand experience. I learned a lot about science and also about myself in science, and how the experiments are done. Even now, I remember some experiments I have done as an undergraduate, and this knowledge is still very useful. After St. Petersburg University, I moved to do my Masters and Ph.D. degrees in Israel at the Weizmann Institute of Science, which was a great experience as well. Living in Israel was exciting; I had an opportunity to travel and see historical places, many of them just unbelievable. Also, the research environment at the Weizmann Institute was great. I moved to do my postdoctoral work in Canada at McGill University, and then moved to the Baylor College of Medicine in Houston. So as you can see, I had an opportunity to travel and live in several different countries, which is great advantage of a scientific career.

My current research interests are related to the mechanisms of human aging and the role of DNA repair and genomic instability in the aging process. As for my outside interests, I enjoy hiking and wilderness camping. I love camping in the north up in Canada, in places so remote that we do not see people for days and can feel at one with nature.

jur: How did you become interested in studying the mechanisms of aging?

Gorbunova: The first time I started thinking about this area of biology was when I was an undergraduate student taking a human genetics course; our professor invited a guest lecturer, and he talked about human cells in culture. He explained that cells taken from human body and put into culture do not divide indefinitely, but stop proliferation after undergoing approximately 60 population doublings. Then, it was calculated that if you take highly proliferative tissues in the human body, 60 population doublings is just enough to provide cells for 120 years of life, which is a maximum human lifespan. I was fascinated by this observation, and it got stuck in my mind. At the Weizmann Institute, I looked around for my Ph.D. project, but no one was involved in aging studies, so I chose to study DNA repair in plants. Then when choosing a field for my postdoctoral studies, (and this is usually the field in which you will stay for the rest of your research career), I remembered the excitement I felt about the puzzle of aging. I find aging fascinating because this is a fundamental biological process, as all living organisms age, and at the same time aging is highly relevant to human health. There are many areas of research that are hard to explain to general public, while aging is something that interests everybody.

jur: Your recent research has to do with the differences in telomerase expression between human beings and mice. Although mice experience tumorigenesis, how often does this occur in comparison to tissue regeneration, and how do you measure telomerase activity in rodents?

Gorbunova: Telomerase is an enzyme that elongates the ends of human chromosomes. When cells replicate, their DNA the very end of the chromosome cannot be completely replicated. To deal with this problem, cells have a special enzyme called telomerase, which helps maintain the very ends of chromosomes. If there is no telomerase around, the telomeres get shorter and shorter with every cell division. This process is actually behind the mortality of human cells in culture, because their telomeres get shorter and shorter, and when they get critically short, the

cells stop dividing. In somatic human cells telomerase is not expressed, and that is why somatic cells arrest their replication after sixty generations. One might wonder why humans don't become extinct; the reason is that telomerase is expressed in our germ cells, so for every new generation, chromosomes have complete telomeres and maintain constant length of telomeres from generation to generation. But in somatic cells, telomere shortening prevents cells from replicating too much when you don't want them to. Such unwanted replication happens in tumorigenesis. Senescence or telomere shortening is one mechanism that arrests cell replication before there's a tumor that has grown too large. This is what happens in humans. In mice, the picture is very different. Mice express telomerase in all of their cells. If you put mouse cells in culture they won't stop after sixty generations: they just continue. Mice in the wild are killed by predators, but if you put them in a protective environment up to 90% die of cancer, while in humans, cancer mortality is about 25%, considerably less. In humans, most cancers develop in the older years past the middle age; in mice, the same thing happens when they are two years old. So obviously the chances of mice getting cancer are much greater. They are missing this important support mechanism of repressing tumor growth: their cells keep dividing. One might wonder why mice do not repress telomerase and do not have the same protection as humans; perhaps there are some benefits of having telomerase expressed. For example, it helps cells divide more actively in case of injury, to quickly regenerate tissue. In mice, it may be more important to have efficient wound healing rather than protection from cancer because cancer is not a big issue in the wild, while for humans it a bigger issue, and it becomes important to protect ourselves from cancer.

We recently completed a very interesting project that deals with this question. We decided to test the common theory in the field that humans repress telomerase activity in somatic tissues because they are long-lived, and mice do not because they are short-lived. We were interested in whether this correlation would be true for a broader range of animals, or if it is really connected to something else, like body mass. Humans are larger than mice, and have many more cells; if every cell can mutate, then humans have a higher chance of one cell becoming cancerous and forming a tumor. So, we took a collection of different rodents, some of which are very long lived (like squirrels, which live up to twenty three years, and beavers, which live up to twenty five years) and others that are short lived (mice generally only survive three years). Interestingly, we didn't see any correlation with life span, but rather with body mass. This suggests that in humans evolved telomerase repression not because they are long-lived, but because they are large. As to how we measure telomerase activity, there are biochemical assays that allow us to do it. We prepare extracts from animal tissues and mix it with radiolabeled primers that resemble telomeres. If there is telomerase in the extract, it will extend the primer, which is then visualized using X-ray film. It was quite an experience to collect the wild animal samples, as it goes beyond the normal lab routine where we purchase mice from commercial vendors - you can't purchase beavers in the same way, so we had to do a lot of collaboration with people from different areas. I am very thankful to my colleagues who contributed animal samples to this project.

jur: Some of your research deals with DNA double-strand breaks. What future do you see for understanding DNA double-strand breaks, and how has characterization allowed you to investigate ways in which DNA repair can be improved?

Gorbunova: Double-stranded breaks are very dangerous types of DNA lesions. It can lead to loss of large chromosome segments, or if repaired incorrectly can lead to chromosomal aberrations like translocations, where fragments are located in the wrong places. These types of aberrations are often found in aging organs or tissues. Chromosomal aberrations are also found in cancer cells. Our laboratory has shown that the process of repairing DNA breaks becomes less efficient and more error-prone with age, so we are now trying to pinpoint exactly what processes lead to this, what proteins are affected, and why it becomes less efficient with age. Once we understand this, we can look for a way to prevent age-related decline of DNA repair, or even to make the repair more efficient, which will help to slow down aging and prevent cancer.

jur: What kind of applications could potentially result from your research or related research, and what kind of impact do you expect to have on your field?

Gorbunova: Ultimately, we would like to extend human lifespan, and this would have an impact on all of society, not only on the aging field; but we are not there yet. Medicine focuses on specific diseases, and many diseases are associated with age, like cardiovascular diseases and cancer. So if we could find out how to slow down the whole aging process, we could at the same time prevent age-related diseases. It would be a global approach where instead of going after each disease individually, we could cure them all of them at once. Some may argue that we don't need more elderly people straining the economy,, which is a huge misconception about the aging field. If we find a way to slow down aging, we would increase not only lifespan, but health span as well, so that people would stay healthy for a longer time. There is no problem with the economy here; people will just keep their active lives longer. Currently in my laboratory, we are looking for ways to counteract age-related genomic instability; as I already said, this would help fight cancer and also help prevent changes in gene expression and other changes that reduce fitness with age.

There is another interesting implication from the telomerase study that I just told you about. We got very fascinated with squirrels, as we found that they have extremely high telomerase activity in their somatic cells. Telomerase activity in the squirrel is about four times higher than that in human cancer cells, and squirrels still manage to live for twenty years without developing cancer. We are now working to identify the mechanisms that protect squirrels from developing cancer despite the high telomerase activity. It is believed that cancer in humans originates from stem cells gone bad. Since stem cells maintain low levels of telomerase activity, it will be very important to find mechanisms that prevent stem cells from turning cancerous.

Another aspect in which we are very interested is how large animals deal with cancer. For example, take a whale: it is huge, and it has so many more cells than humans. Every cell has a chance of getting a mutation and becoming cancerous, so you

would expect a whale to get cancer pretty quickly; yet whales live for a hundred years or so. Perhaps they have other tumor-suppression mechanisms that even humans don't have, and it would be nice to find out what these mechanisms are and whether they can be activated in humans.

We're also interested in the comparative aspects of aging. For example, what makes a mouse short-lived while a squirrel is long-lived? Understanding the mechanisms that determine longevity of the species would help to manipulate human lifespan.

jur: How can undergraduates get involved in research in your field? What can they expect? Do you have any advice for up-and-coming biologists?

Gorbunova: I must say that I enjoy being at the College Biology Department. In my previous position at the Baylor College of Medicine, there were only graduate students around, and it was a little less vibrant than here. Every semester two to four undergraduates work in my lab on independent research projects; and they contribute significantly to our research. There are also summer opportunities, like Reach funding, DeKieweit fellowships, the GEBS summer program, etc. So there are many ways undergraduates can enter the field. It's very exciting to have enthusiastic young people around.

What can they expect when entering the field? Expect to have a lot of fun, because research is a lot of fun. They will be involved in research in an active laboratory using the most modern techniques. In the aging field, they will be working on problems that have relevance to medicine and basic science at the same time. In our laboratory, we do a lot of cloning; we culture human cells, and we even collect samples from wild rodents, so there is some field work as well. In general, research is a very exciting field, and I enjoy every minute of it. Even for me, it's sometimes strange to call it a job because a job is presumably something tedious, but what you do in research is so exciting, unlike a typical job. It is amazing we're getting paid for what we do.

What I love about biology is that you can ask very basic questions about life, and at the same time it can be very useful in applied medicine to make people's lives better. Biology is a rapidly developing field that is now on the rise and tremendous resources are being put in it, so if you chose biology you made a wise choice. For students starting research, my advice is to be prepared to learn, because we always learn new things. Be prepared that some of your experiments won't work right away; there will be some disappointments, but overall, research is extremely rewarding.

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