Journal of Undergraduate Research



University of Rochester

Volume Four Issue Two Spring 2006

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

hy should undergraduates engage in research?

This question has been earnestly raised, both to us directly and more generally in academic atmospheres. It is undeniably true that undergraduate research often goes unfinished, and is gravely lacking in one respect or another even when complete. Sometimes the problem is a lack of follow-through on the part of the researcher, notwithstanding the afforded an immense structure undergraduates are often afforded in which to pursue the otherwise generally solitary task of research. More often, however, the final product suffers from a lack of knowledge, perspective, and experience during the question formulation process. After all, quality of answers found is limited by quality of questions asked, both at the outset and along the way.

So again, why should undergraduates engage in research? The first answer is obvious – one cannot ever hope to improve without practice. Everyone, even the most brilliant, must start somewhere, must begin ordering their questions and analysis at some unskilled point. For most, the first time such questions are seriously confronted is during their undergraduate years. Having structures and institutions in place to guide and assist in this process only makes sense. Furthermore, we can all think back to the mistakes we have made in our own lives and realize that they have been just as valuable (if not more so) than our successes in shaping later methods of inquiry. Very few are able to fashion motivation from successes equal to the motivation failures provide.

But there is something more beyond the 'training wheels' argument for undergraduate research. Think of the excitement associated with brushing with discovering a new and fascinating phenomenon – there is a cavalcade of new perspectives, new questions, and new methods of inquiry. As time goes on these thoughts are tamed and ordered to permit thorough investigatopm, but in the process they can become ossified. Excitement and novelty are harbingers of innovation, and with taming and directing can produce whole new lines of inquiry. Not all undergraduate research, of course, necessarily produces such novel questions, nor do all undergraduate papers even contain a nugget that may eventually be developed into a revelatory new question. But that potential is most certainly there, and uncovering it is the mission of educators and students, and *jur* as well.

Sincerely,

Trika Haga

Samuel Boyer

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Editors-in-Chief





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Perspectives on Research

jur interviews Terry Platt, Ph.D.

Dr. Platt is Professor of Biology, with a joint appointment in the Department of Biochemistry and Biophysics at the University of Rochester.

jur: Could you tell us about your educational and professional background and experience?

Platt: I grew up in Chicago; my father was a physicist on the faculty of the University of Chicago. I went to Swarthmore College for my first two years as a physics major, but I decided to transfer to the University of Chicago. I had to change my major to mathematics, because that was the only major I could transfer to and I figured it would be good for me no matter what I did, and I enjoyed Chicago a lot. At that point, I ended up going to the Peace Corps for two years, teaching math and physics to African high school students. We were trained in New York City, and while I was there it turned out that I learned something about teaching, because their job is training teachers, so we got really good training. My father has always been on the fringes of and interested in molecular biology, and when I was in the Peace Corps he sent me a copy of Watson's book, Molecular Biology of the Gene. And eventually I found myself at Harvard for graduate work on the lactose repressor with Wally Gilbert and Klaus Weber. For my subsequent postdoctoral research at Stanford, I joined Charles Yanofsky's research group of around fifteen scientists, all of them initially working on different areas of the tryptophan operon: Some were doing genetics, some enzymology, some biochemistry, a few were doing gene regulation, and some were sequencing DNA or RNA. Over the three years that I was there the group made some breakthrough progress, and it was great to be part of all that. Then I joined the faculty at Yale and was there for ten years, before coming to Rochester.

jur: What kind of research did you do in graduate school?

Platt: I worked on the lac repressor molecule, which is a tetrameric protein that binds to a site where polymerase initiates transcription of the lactose operon, encoding the enzymes responsible for the metabolism of lactose. In the absence of lactose this repressor binding prevents that operon from initiating transcription of its messenger RNA. I worked on the structure-function relationships in the repressor and, for example, was able to show that if we treated it gently with trypsin we cleaved off part of the molecule that turned out to be the DNA binding part, though it still bound the inducer molecule perfectly well. It was a very exciting time to be a graduate student.

jur: When did you first get involved in research? How have opportunities for undergraduate involvement in research changed since then?

Platt: Between my sophomore and junior years I got a summer job with the Woods Hole Oceanographic Institution. The next summer I worked at Scripps in La Jolla, where we did some hypothesis-directed research to understand diving mammals and physiology. It's hard to answer the question about changing opportunities, because I was at different schools than Rochester, but in general, all around there are huge opportunities that I didn't have. At the University of Rochester, we take pride in our undergraduate research opportunities. I would certainly say that here it's generally very easy to get involved, though you often have to start out by cleaning glassware and preparing media. If you go to lab meetings, people will know faculty members you can get involved with.

jur: What kind of research have you been involved in over your career?

Platt: There were two major areas that I got involved in. One stemmed directly from my post-doctoral work, and gave some interesting results that were separate from my postdoctoral involvement in the attenuation of transcription, though that gave me the tools for looking at signals at the end of the messenger RNA. I've always been interested in gene regulation and loved the idea of all the different varieties of transcriptional control at the promoter end, namely repressors or positive activators. But I thought, "Well, there are many people out there with established labs, and if I go out there and try to compete in the currently hot arena I'll get killed, unless I'm stupendously lucky or brilliant (neither seemed likely)." Then it occurred to me, "Maybe the important thing to do is to carve out my own niche that nobody's thinking about too much right now but might be really important in five years." And so, that is exactly what I did. Since I had already been working with messenger RNA sequences at the end of the tryptophan operon, it seemed natural to investigate how this molecule

(RNA polymerase, which copies DNA into RNA) knows where to stop. It's the same question as if you were studying sentence structure: how do you know where to start? You start because there's a capital letter at the beginning of the sentence. How do you know where to stop? You stop when you get to a period. At a colon or a semicolon or a comma you don't stop, you pause. The problem is one of genetic punctuation, and I chose an aspect of punctuation that was not already well-established and crowded. As I got interested in this area, I started working in yeast as well, and as a consequence of a sabbatical with Ira Herskowitz at UCSF I learned enough lore for that part of my research effort. Initially I was interested in the transcription termination of E. coli. The big problem there is that my earlier work was with lower eukaryotes rather than prokaryotes, and there are enough differences between the two systems that made introducing many of the same techniques that I value more difficult. That soon devolved into a really different area of the same general problem, which is that messenger RNA in eukaryotes is in fact processed. This masked anything that we could imagine to try to find out about termination, but it turned out not very much was known about the process of RNA 3' end formation, so we pursued that instead. These two big areas of transcription termination in prokaryotes and messenger RNA 3' end formation in yeast have really comprised most of my work.

jur: What makes the rho utilization (rut) site what it is?

Platt: We simply don't know. My laboratory group and many other laboratories have tried for years to figure out what it is about this particular region in bacteria that confers the ability to terminate transcription. Every hypothesis that we've thought to test has failed. There are some ideas in the literature, and we know that cytidine residues are important; but if I had to guess, I would say that we don't know enough to ask the right question. It's lurking there, but I would guess that we haven't yet asked the right questions. It's like self-splicing RNA molecules, before that was accepted as "real" - researchers spent a decade trying to purify the protein factors that they were sure had to be in there to carry out the splicing. So thoroughly was everyone imbued that RNA can't be an enzyme that they kept trying to purify their RNA more and more and it still kept doing the splicing reaction, albeit very slowly, and that kind of fit in with their prejudice that there had to be a really tiny contaminant there doing it. And, so, back to your question, the answer to the rut site is that we don't know, and if I had to guess I'd say there's some kind of sequence or structure there that is being formed, possibly in conjunction with one or more other proteins, and that it doesn't happen until the right circumstances are present. We've tried all the sequence bashing that we can do, and it hasn't given us an answer. So the point is that we need to know more information before we can do an experiment that tests this hypothesis. The concepts don't exist. We're just missing one critical piece of the puzzle, and as I say, if I had to guess, it would be in the realm of RNA structure.

jur: What is the role of rho protein and what experiments are you performing to discover more about it?

Platt: Rho protein acts at these mysterious sites, and we don't know how it acts. Let me tell you a true story; this is one of the big breakthroughs we had in my lab. It had been known for some time that rho factor has an RNA-dependent ATPase activity. Our idea was somehow that the hydrolysis of ATP would help it catalyze transcription termination by the bacterial polymerase. I remember very clearly our breakthrough on its function with this site. I was at a Gordon Conference; these are summer scientific conferences with between 100 and 200 scientists. A lot of the great moments at the Gordon conferences happen outside the lecture halls, where people go for hikes in the woods or sit around the bar in the evening and talk science or go sailing and talk science. One day at lunch I was sitting with Howard Nash from the NIH and he said, "Hi Terry, how are things going? What's new with rho factor?" So I said, "Well, we're trying to figure out how it works. The puzzle is that that we know it can hydrolyze ATP, and we think that we know there is an elongation bubble, where the DNA is separated and the RNA is base-paired with part of the bubble and somehow the rho factor bound to the RNA has to be able to separate this bit of RNA, the last 10-12 base pairs that's duplexed to the DNA, which is about one turn of the helix." And as I said "helix", I thought to myself, "That's it! Rho has to be a helicase, because its job must be to unwind that helix and separate it from the DNA strand!" So I went back to my lab, very excited. We did the first experiment in a week to the test the hypothesis by making an artificial duplex with a piece of RNA that we know would be a substrate for rho, and annealing it to some single stranded DNA, to form a duplex region, of some 20-30 base pairs. It is amazing to be able to come up with a hypothesis in the middle of a sentence and to go back to your laboratory and test it; and we found out that in this case it was right, based on the control. That happened a long time ago, and a big difficulty in the interim has been that we haven't learned a lot more about rho factor in the intervening years. Another question is how the system I just described, which is independent of RNA polymerase, actually coordinates its action with RNA polymerase to get it to terminate.

jur: What kind of research is going on in your field at the University of Rochester, and how can undergraduates get involved?

Platt: There is a great deal of research going on at UR; in immunology, microbiology, biochemistry, genetics, and so on, an enormous variety of projects. The best way to get involved with any of those is to talk to a faculty member who has gotten



you interested in something. Also, talk to any of your friends who are working in laboratories and find out what they like and don't like. You can also check the departmental or faculty websites and rosters to find out who is doing research in various areas. At that point, you just start knocking on doors, looking for possible jobs in the labs.

jur: The workshop approach to education at the University of Rochester is well-known to students here. Could you tell us about its history, particularly with your own biochemistry classes?

Platt: There's a problem in high school because you're encouraged to be your own study person, and you're often not supposed to work together - students are taught that they must answer problems on their own. And so many people were pointing out that this is not a great way to work. Indeed, if you think about the real world after college, in what arena does anyone have a job where you're not supposed to talk to anyone else about what you're doing, or get other people's advice, or brainstorm? That's basically the origin of this. And at the University of Rochester, Vicki Roth, the director of Learning Assistance Services, came here around 1990 and did some pilot studies. Then in 1995 she and Jack Kampmeier came up with a grant proposal and got money from the NSF that helped actually set up and implement the workshop model here. When I was starting to teach the junior level biochemistry course I thought workshops could be very beneficial. So I asked Vicki Roth about setting up workshops in biochemistry. And so we talked about it, and she gave a lot of advice and then helped get it started. I think it's a great complement to a lecture course because it really gets students involved and working together. The value of workshop is that there are three things you are going to be asked by future employers once you leave college: Are you a critical thinker? Do you know your subject matter? Do you work well with other people? They want to know whether you have engaged in synergetic activities where the work that comes out of the group is greater than the sum of the abilities or skills of the individual members.

jur: How does the workshop system help students make their way through difficult subject matter and better understand the material?

Platt: I think the workshop helps in a number of different ways. One way is that it shows everybody that they're not alone in their difficulty mastering the subject. But the other side is that it generally helps people in showing them the material from a variety of viewpoints; it helps them in organizing the material as well. I want to add that one of the areas of research that is going on at the University of Rochester is research on education. This is a study that I just passed out based on my workshop experience in biochemistry, after I'd been teaching the course for three years, in which we actually asked whether workshops are working at all, and how we know. And the answer is that yes, they are helping. Students who attend workshops do better than those who do not. That's the area of research I am currently involved with now. I study the effects in variation in workshop design that will be beneficial to students. And I certainly think that the University of Rochester is in the forefront of institutions with respect to this area of research. I think the answer to the last part of is that medical schools, in

particular that of the University of Rochester, have Problem Based Learning (PBL) as an integral part of the curriculum. In fact, this is the way that real physicians and real scientists work. You get together once a week and you talk about the problems – research or medical – that you are working on. As a University of Rochester undergraduate it is very useful to have this sort of thing available.

jur: What direction do you see the domain of research going? Do you feel that some of the ethical debates raging between the political and scientific communities are impeding medical research?

Platt: I think the major impediment is a lack of understanding of what science really is, and the idea that hypotheses are there to be tested. Faith-based initiatives are fine, but they cannot be confused with science. People can believe what they want but if you want to prove that something is true or not true, you should always be prepared to propose and conduct experiments to test your hypothesis. And for sure, politics is always going to impinge on various aspects of science; sometimes it will end progress, but there are checks and balances imposed, but sometimes they can be a good thing.

jur: How does research develop a student, as opposed to rote memorization and reading?

Platt: I think there are two or three parts to that. One is, getting back to the idea of hypothesis testing and the methods of strong inference, that if you start applying those ideas to critically finding out the truth rather then being persuaded by an idea and then trying to fit the facts into what you're looking at, it's certainly a benefit - most research you get involved in will force you to do that. But I think more importantly, the moment you start doing research of your own, you create interest because of the ownership component that is really exciting and attractive. And part of the excitement, which goes hand and hand with frustration, is that when you're doing research you're trying to do something that nobody in the history of the world has ever looked at before, and if the answers were to emerge easily then you wouldn't be doing it because somebody else would have done it long ago. The cutting edge of research is at the same time a very exciting area and a very frustrating area - you have to be prepared for that, and you have to like the process of research and get away from the idea that you're only in it for the answers, because the answers are often a long time coming. Finally, it's healthier overall if you can be satisfied with small but steady incremental advances rather than always striving for the big scientific coup. You can't push the river, but you can steer the canoe...

Monte Carlo Lattice Simulations of Biochemical Reaction Mechanisms

Tom Weingarten, 2006

Advised by Mitsunori Ogihara, Ph.D. and Joel Seiferas, Ph.D., of the Department of Computer Science, and Professor Harry Stern, Ph.D., of the Department of Chemistry.

raditionally, experimental assays designed to study the rate laws of biochemical reactions have been carried out in vitro. The rate laws derived from such experiments hold only so long as do the assumptions on which they are based, chief amongst which is the assumption that the reaction conditions in vitro are analogous to those in vivo. If the laboratory reaction environment differs significantly from that of the cell, then we must rethink rate laws we have based on the results of these experiments. Recent research has suggested that for many reactions this may be the case. In particular, the law of mass action has been challenged by many authors as being unlikely to hold within the constraints of the cellular environment.^{1,2} Firstly, the law fails because the system is not dilute. Studies have found that the macromolecular concentration of the cell (the concentration of the largest class of molecules, including proteins, in the cell) is 50-400 mg/ml, and as much as 5-40% of the total volume of the cell can be occupied by macromolecules.^{3,4,5} Additionally the viscosity of the mitochondrial matrix, the interior of the mitochondria, can be as much as 25-37x higher than that of a traditional experimental buffer solution.6 Secondly, the medium can not be said to be well mixed, since macromolecules can enter the cell at multiple locations specific to the type of macromolecule, and the diffusion of these molecules once in the cell is severely impaired.7 In the cytoplasm, macromolecules can diffuse as much as 5-20x slower than in saline solutions.^{8,9,10} Furthermore, it is understood that the cell is a highly compartmentalized system, where many reactions are spatially confined to specific organelles.11 In fact, without this compartmentalization many reactions would not be able to occur at all. Still more reactions are confined to two dimensional surfaces, such as lipid membranes, or one dimensional environments such as cytoskeletal pathways. Because of these factors it is questionable whether the law of mass action holds within the cell.

It has been proposed that the conditions of the cellular environment may cause reaction mechanisms to exhibit fractallike kinetics. The kinetics can be explained by the theory that the cell contains a percolation cluster (a set of connected lattice sites which touches the top and bottom or left and right of the lattice) of intra-cellular elements such as lipid membranes or cytoskeletal structure.^{12,13,14} Percolation theory tells us that the fractal dimensionality of a system varies with the degree



to which it is crowded by obstacles. As the level of crowding approaches or reaches the percolation threshold, the formation of percolation clusters is observed. In the case of this simulation our percolation clusters will be clusters of lattice sites which are unoccupied by obstacles. These clusters affect the random diffusion of macromolecules through the system and cause the reaction mechanism's rate law to exhibit a time-dependent factor. While the Euclidean dimension of the reaction, either one, two, or three dimensional, is relatively easy to simulate in vitro, the conditions leading to fractal dimensionality are significantly more difficult to reproduce.

Although intracellular conditions are difficult to duplicate in the laboratory, computational simulations offer the ability to observe fractal-like kinetics. Computational results by Berry and Turner and Schnell have both provided evidence to support the theory that fractal-like kinetics are observed when obstacle densities approach the percolation threshold.^{15,16} Similar simulations have been tested against pharmacokinetic data with success.¹⁷ Studies with an enzymatic glucose oxidation reaction have also shown correspondence to the simulation results, and further studies using fractal techniques to predict biological processes have also met with success.7,18 These studies are not enough to verify the accuracy of the proposed simulations, but they provide clear evidence that the theories of traditional kinetics, based on the law of mass action, need to be revisited. The need for further studies comparing computational and experimental results is still great, and it will be necessary in the future to verify the proposed models. Experimental studies are, however, outside the scope of this paper. The focus is instead placed on the improvement of current computational techniques and furthering the analysis of their results.

Methods Simulation Definitions

In this section we will lay out the theoretical basics of the simulation. For a more detailed description of how the simulation was implemented see section "Implementation." The simulation takes place on a two dimensional rectangular grid space. Each grid space can be either empty or occupied by a single molecule. No grid square may be occupied by multiple molecules at any given time step. Each molecule is defined by its coordinates in space and its associated "type". A molecule



Fig. 1: The simulation program can pipe its output to a Java program which displays a graphical output of the simulation in progress. The image on the left is the display without any modifications. The image on the right is the display after the simulation has been reduced to the largest cluster problem by removing all the molecules which were not in the largest cluster.

type is defined as a 3-tuple $T=\{R, I, S\}$ where R is the set of reactions which contain member T as a reactant, I is a boolean (0 or 1) value which represents T's mobility as either mobile or immobile, and S is a natural number representing the molecule type's speed (probability of movement) as a factor of 1, where 1 means the molecule moves every time step (S=n implies a molecule of this type has a probability of 1/n of moving on a given time step). A reaction is defined by a 3-tuple $R=\{M_p,$ M_{p} , f} where M_{p} is the multiset (the set with multiplicity, so the number of each element is significant) of reactant molecule types, M_p is the multiset of product molecule types, and f is the forward reaction rate. Note that the reverse reaction rate is not part of the tuple. If a reverse reaction occurs it must be defined as a separate reaction, such as $R_r = \{M_r, M_R, r\}$, where r is the reverse rate. A "spontaneous" reaction, such as one of type A \leftrightarrow B+C, is represented as a reaction where $|M_p|$ (meaning the number of molecules) is equal to 1.

Simulation Rules

The simulation can be run for an arbitrary number of time steps. At the beginning of a time step $t \ge 0$ a molecule m is chosen randomly from the set of all molecules in the system. A direction is then chosen randomly from the 4 neighboring grid spaces, and the molecule attempts to move to that grid space with the following rules.

(1) If the grid space was formerly unoccupied, molecule m now occupies that space, and the grid space which m formerly occupied becomes empty.

(2) If the grid space is occupied by molecule m_1 , and there exists no reaction which contains both m and m_1 in its multiset of reactants M_R , then m remains in the same grid square.

(3) If the grid space is occupied by molecule m_1 , and there exists a reaction R_1 which contains both m and m_1 in its multiset of reactants M_R , then the location which is occupied by m_1 is chosen as the site of a possible reaction. First a check is done to see if there are $|M_p|$ empty spaces in the 5 spaces including the site and its neighbors. If there are not, no reaction takes

place, since it would be impossible to fit the products of the reaction on the grid. If enough empty spaces exist, a number a is chosen uniformly at random from the interval [0,1). If a < f, where f is the reaction rate of R_1 , then a reaction occurs. First, both molecules m and m_1 are removed from the grid. If $|M_p|=1$, a new molecule of type x, where x is the only member of M_p is placed at the reaction site. If $|M_p|>1$, a new molecule of type x_i is added to the grid for each element x_i of M_p . The location of these new molecules is chosen uniformly at random from any unoccupied members of the reaction site and its neighbors. Both new molecules are marked as "inactive" for the remainder of the current time step. Inactive molecules can not react with any other molecules that collide with them and do not move. At the end of a time step, all inactive molecules lose their inactive marking.

Implementation

The initial implementation of the algorithm was written in Java and included a graphics module to display the contents of the grid as the simulation occurs. For speed reasons the program was rewritten in C. The output of the C program can be piped through a Java program which displays the output graphically, as shown in figure 1. The graphic display has proven extremely useful for debugging purposes. It is important to note that although this paper only explores the use of this program for simulating the Michaelis-Menten formalism, it is flexible enough to simulate almost any reaction mechanism by altering the input script. All of the experiments listed in this paper were carried out without modifying the source code, only the input script provided to the program changed for each simulation. (The only exceptions were edits to the data-reporting module of the program, which was amended to print special data in a few cases.)

Data Structures

The key to the simulation program's ability to simulate many reaction mechanisms lies in its data structures. The data structures used for the simulation were chosen to maximize efficiency without sacrificing flexibility. Figure 3 shows the relationship of the data structures used by the simulation



Fig. 2: A diagram of the data structures implemented in the simulation program. Boxes represent data structures, arrows represent pointers.

program. The molecules are stored in a two dimensional array, which represents the lattice being simulated. The molecules are also threaded through one of two linked lists, depending on whether they are mobile or immobile. Each molecule stores its own type in an integer and its location as a pair of integers. The integer representing type is an index to an array of all the possible molecule types. Each molecule type (eg. Enzyme, Substrate, etc) contains a flag setting molecules of its type as either mobile or immobile and an integer describing the molecules' speed. A speed of 1 means a molecule type moves every time step, a value n > 1 means the molecule moves with probability 1/n on each time step. Each type also includes an array of pointers to linked lists. The array size is equal to the number of molecule types in the system, and the linked list pointed to by the array element at index i represents all the reactions that contain the molecule type indexed by i and the molecule type which contains the array. Each element in the linked list contains a pointer to the next element in the list as well as a pointer to a reaction. A reaction is represented by a data structure containing an array

of pointers to the reactant molecule types, an array of pointers to the product molecule types, and an integer representing the probability of the reaction occurring upon the collision of the reactants. (NB: A reaction which produces two or more molecules of the same type will have a product array with a number of pointers to that molecule type equal to the number of molecules of that type to be produced. The same is true for reactants without loss of generality.)

Simulation Functions

After reading input from the user, the program begins a loop which iterates for a specified number of "ticks" (time steps). On each tick, the program performs the following actions:

(1) At the beginning of each tick, the program checks a variable to see if the current molecule array needs to be synchronized with the linked list of mobile molecules. The simulation begins with this variable set to true, so on the first run the program will always call the synchronization



Fig. 3: Recreation of Berry's simulation carried out to 600 time steps. Same simulation continued to 10,000 time steps. The grey line represents the point at which Berry's simulation terminated.

function, and then set the variable to false. After the first tick this function is only called as needed. This function is O(n). It allocates an array of size n, where n is the number of mobile molecules in the system. It then copies each element of the linked list into an array position. As it traverses the linked list, it also checks for molecules which have been marked as "dead." These molecules are removed from the linked list and not added to the array. The more perceptive reader may notice that this leaves us with an array allocated with more elements than there are molecules in the system. This is avoided by not counting "dead" molecules when the value of n is decided. It is also important to note that this function could be optimized by removing the linked list entirely and replacing it with two new linked lists, one of molecules which have been added since the last tick, and one of molecules which have been removed. We chose not to implement this because studies showed it made only a very small speed increase, and having a full linked list of all the mobile molecules is useful for some other functions.

(2) In order to simulate random molecule movement, the molecule array is now ordered with a uniform random distribution. This function is also O(n). The program sets variable a = n - 1, where n is the number of elements in the array, and sets r to an integer chosen uniformly at random, such that $0 \le r \le a$. The array elements at a and r are then swapped and a is decremented by 1. This process is continued until a = 1.

(3) The program now beings a loop which executes once for each molecule in the array. First, two checks are performed to see if the current molecule is not "dead" or "inactive." If it passes these it chooses a number r uniformly at random such that $0 \le r \le s$, where s is the speed factor of the molecule's type. If $r \ne 0$, this molecule does not move, and the program repeats this step with the next molecule in the array. If r = 0 we continue to step 3a.

(3a) If the molecule's type has any spontaneous reactions (see Simulation Definitions), we choose a random number to see if they occur. If the molecule does not, we continue to step 3b. We choose a new random number, r_1 such that $0 \le r_1 < c$, where c is an arbitrary constant. We then enter a loop which repeats once for each spontaneous reaction the molecule can engage in. If $r_1 < cp'$, where p' is the probability of the first

spontaneous reaction occurring, then that reaction occurs. We repeat this for each spontaneous reaction, incrementing p' by the probability of the current reaction each time. If any reaction occurs jump to step 3c, otherwise continue to step 3b. This method is chosen since the reaction probabilities are assumed to be independent.

(3b) The program first chooses a random direction for the molecule to move in from the 4 cardinal directions. If the grid space in that location is not occupied, the molecule is moved from its current grid space the space in the chosen direction. If the grid space in the chosen direction is already occupied, and the molecule is not marked as "inactive," the program attempts to find a reaction between the current molecule and the occupying molecule by traversing the list of reactions which the current molecule type shares with the occupying molecule type. If there are any such reactions, they are each checked in a manner similar to step 3a. If there are none, the current molecule does not move, and we continue from step 3 with a new molecule.

(3c) This step is only reached if a reaction occurs. In order for any reaction to occur, there must be enough space for the products to be placed, so the program checks if the current grid space and its immediate neighbors contain enough unoccupied spaces to place each product in a unique location. If not the reaction fails and the program returns to the step which pointed it to this one, proceeding as if no reaction had occurred. Otherwise the reactant molecules (molecule in the case of a spontaneous reaction) are removed from the grid and marked as "dead." New molecules are created and added to both the grid and the molecule linked list appropriate for their type's mobility. The newly created molecules are marked as "inactive" so as to prevent them from reacting with other molecules this time step. The program then continues to step 3 with the next molecule in the array.

Results Rate Law

The most significant result of the experiments which were conducted using this simulation is the creation of a new rate law. Our simulations suggest that the new rate law is significantly more accurate than those which were previously used to model the reaction kinetics. Kopelman originally suggested the fractal-



Fig.4: A comparison of the Zipf-Mandelbrot distribution and the combined power-exponential law. The blue points represent the simulation data provided by the recreation of Berry's simulation to 10,000 time steps. The yellow line represents the Zipf-Mandelbrot distribution. The green line represents the combined power-exponential law proposed in this paper.

like kinetics formula for reactions of type A+B $\rightarrow \theta$:¹⁹

$$k(t) = k_0 t^{-h} \quad t \to \infty$$

Schnell and Turner later proposed that the Zipf-Mandelbrot distribution was a more accurate description of the rate law:

$$k_1(t) = \frac{k_1^0}{(\tau+t)^h} \quad 0 \le h \le 1$$

The results from which Berry and Schnell and Turner drew their conclusions regarding the equation for the reaction rate were based on simulations which ran for only 600 time steps. We reconstructed their simulations within our own program and allowed our simulation to run for 10,000 time steps. When fitting our data to the two previously discussed equations, an interesting observation was made. As shown in figure 3, the data seems nearly linear (on a log-log plot) when examined to only 600 time steps. From this graph we could easily infer that the rate law is a power law. However when the same simulation is run to 10,000 time steps the linear relationship becomes decreasingly valid. After around time step 100 the data begins to look increasingly less linear on a log-log plot and more linear on a semi-log plot. In accordance with this finding we decided to attempt to fit the data to an exponential function. This performed better than the fractal-like law, but worse than the Zipf-Mandelbrot distribution.

From the log-log and semi-log plots of the data it seemed possible that the reaction may occur in two phases. In fact, simulations in which the reaction was slowed down as much as 100x have shown that the power law representation is nearly perfect for the short time scale. In light of these findings we propose yet another mechanism, which, throughout the experiments listed in this paper, fit the data significantly more accurately than the two mentioned above:

$$k(t) = \frac{k_0}{(\tau + t)^h e^{at}}$$

We will refer to this new rate law as the combined powerexponential law. Figure 4 shows the actual data plotted beneath the fitted curves. The Zipf-Mandelbrot and combined powerexponential laws both fit reasonably well to the center portion of the curve, but the combined power-exponential law is significantly more accurate at very short and very long time scales. The r^2 value of the fit is .99 for the combined law, and .96 for the Zipf-Mandelbrot distribution. In other simulations, with different initial values of [S] and [E], the fit of the Zipf-Mandelbrot distribution dropped as low as .935, while the combined law's worst-case r^2 -value was 0.991.

Reactant Segregation

The rate laws proposed by Berry and Turner and Schnell have a time-dependent factor. This dependence is the result of the anomalous diffusion of reactants over time.²⁰ The anomalous diffusion exhibited by random walkers on a percolation cluster causes reactant segregation, where enzymes and substrates become further apart on average over time. The increased distance between reactants causes a decrease in the number of reactants which are within sufficient proximity to react with one another, thus lowering the rate constant. It would therefore be more accurate to describe our rate law as a function of diffusion than as a function of time. However since diffusion itself is dependent on time, and time is far simpler to measure, we choose to define the rate law as a function of time for the sake of practicality. This does not imply that understanding the effects of anomalous diffusion is not worthwhile, in fact, quite the opposite is true. For instance, an understanding of anomalous diffusion is important when predicting what effects will be caused by the addition of new molecules to the simulation after it has already begun, as is done in the simulation conducted by Lin et. al. In such a case diffusion is not only dependent on time, but is also affected by where and when new molecules are added. Clearly a time-dependent rate constant will not suffice in this case. In a broader sense, a firmer understanding of the effects of anomalous diffusion may lead to a generalized deterministic rate law for multiple reaction mechanisms, not just the Michaelis-Menten formalism studied in this paper. In order to further investigate these effects, a function was created within our simulation program to study the distances between S and E molecules at different points in time. We define the "average shortest distance" by the following formula:

$$\overline{D_S} = \frac{\sum_{i=1}^{c_E} \min(D(E_i, S_1), D(E_i, S_2), \dots, D(E_i, S_{c_S}))}{c_E}$$

We define the "average average distance" by:

$$\overline{D_A} = \frac{\sum_{i=1}^{c_E} \sum_{j=1}^{c_S} D(E_i, S_j)}{c_E}$$

Where c_{E} is the count of enzyme molecules in the system, c_s is the count of substrate molecules, E_i is the i-th enzyme molecule (using a 1-based index), S is the j-th substrate molecule, and D(E,S) is the shortest number of moves in which E can reach S without passing over an obstacle. An important distinction is made here between the calculation of distance as the minimum number of moves necessary to reach the co-reactant without passing through an obstacle, whereas previous papers have measured the root-mean-squared displacement between the two, regardless of obstacles. We chose to measure distance in this way because it better captures the distance within the percolation cluster of the two molecules. The calculation of $D(E_i, S_i)$ allows E_i to pass over other mobile molecules in its movement. This assumption is reasonable since other mobile molecules slow movement by a constant factor over the entire accessible lattice. The specific locations



Fig 5: [A] The histograms of D_s at steps 0, 50, 100, 500, 1000, 2000, 5000, and 7500. [B] The change in \overline{D}_s (y-axis) over (x-axis). Both of these are the results of averaging over 250 runs of the recreation of Berry's simulation carried out to 10,000 steps.



Fig. 6: [A] The histograms of D_A at steps 0, 2000, 5000, 7500. [B] The change in \overline{D}_A (y-axis) over (x-axis).



Fig. 7: [A] The histograms of D(E₁S₁) for $0 \le I \le c_{E}$, $0 \le j \le c_{S}$ at different steps. [B] The change in $\overline{D_{S}}$ (y-axis) over (x-axis).

of these molecules at a given point in time is irrelevant over the mean field approximation. The validity of this assumption breaks down if we introduce molecule types which move more slowly than E, since slow moving molecules can act as obstacles over a short time slice. The validity of the assumption is also degraded if we have a reaction mechanism which produces more or less products than it consumes reactants, since the changing concentration of mobile molecules in the system will alter the factor by which diffusion is slowed over time.

In one step any single enzyme molecule can only react with a substrate within two moves of it. Therefore it can be assumed that the rate law is probabilistically proportional to the number of substrate molecules within 2 steps of any enzyme at a given time step. The probability of such a reaction occurring is related to the initial positions of the molecules relative to each other, the order in which the molecules move, whether they move towards each other or away from each other, and the existence of other molecules surrounding the potential reactants. Although a detailed probabilistic analysis was not possible within the frame of this research, a rough estimate places the probability of reaction at around $0.25S_1+0.0625S_2$,

where S_i is the probability of a substrate molecule existing within i steps of a chosen enzyme.

The quantities of D_s and D_A were measured by the use of a breadth first search on the grid space. For D_s the search halts after finding the first substrate, whereas for D_A the search continues until the entire accessible space has been exhausted. The values of D_s and D_A are then calculated by repeating the search for each enzyme molecule and averaging the results. Figure 5 shows the results of the breadth first search for D_s and figure 6 shows the results for D_A . A third histogram, shown in Fig. 7 was created by taking the value of $D(E_i,S_j)$ for $0 \le I \le$ c_E , $0 \le j \le c_S$. This histogram can be interpreted as f(x) = the number of paths from an enzyme to a substrate of length x.

The histograms in all three sets fit well to a beta distribution, suggesting a possible cause for the exponential behavior seen in our rate law. In simulations run without obstacles the histogram fits a triangle distribution with a constant width and an exponential decrease in height over time. For early steps the histogram of $D(E_i,S_j)$ shows an interesting deviation from the beta distribution up to around x = 30, which is likely related to the power law relationship exhibited early in the simulation.

As time progresses the histograms begin to fit better to the beta distribution and the effects of the deviation become less evident. This observance is predicted by the disappearance of the power relationship in our rate law over time.

Discussion

The results presented in this paper provide clear evidence that the rate law, at least under our simulated conditions, is more complex than previously believed. The simple powerlaw formalism does not fully capture the behavior of the function, whereas the proposed combined power-exponential law provides a more accurate model. Preliminary results indicate that the explanation of this effect may lie in reactant segregation, however a more rigorous mathematical analysis is necessary before this conclusion can be affirmed. A probabilistic analysis of the dependence of the frequency of reaction on the reactant segregation (or more accurately described as variables x and y, the number of reactants within distances of 1 and 2 respectively) might help to understand the relationship between anomalous diffusion and reaction rate.

It is also important to note that there are many factors our simulation does not take into account. For instance, all of the macromolecules are assumed to be of the same size. In practice this is not always the case, and it is likely that a continuum of various sized molecules interacting within the same area has an additional effect on the reaction kinetics. Furthermore, it is well known that many macromolecules within the cell have a enzyme mediated tendency to aggregate and form larger structures. It is likely that this will further complicate the results of crowding on the reaction mechanism.

Acknowledgments

The author would like to thank his thesis committee: Professors Mitsunori Ogihara and Joel Seiferas from the University of Rochester Department of Computer Science, and Professor Harry Stern of the Department of Chemistry. Their advice and input to the project was indispensable. In addition many thanks are due to Hugues Berry for his original work in creating the simulation rules, as well as providing the data from his simulation, which was used to verify the program described in this paper. The Lyx editor was used to write this paper and XFig and Gnumeric were used to create the figures. Grace was also used to plot functions.

The Gnu Scientific Library (www.gnu.org/software/gsl/) was used to perform nonlinear data fitting. The GSL uses the Levenberg-Marquardt algorithm as implemented in the scaled LMDER routine in MINPACK. Minpack was written by Jorge J. Moré, Burton S. Garbow, and Kenneth E. Hillstrom.

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About the Author

Tom Weingarten will receive his B.S. in Computer Science from the University of Rochester in May 2006. After graduation he plans to get his Ph.D. in Computational Biology. This paper was presented as his honors thesis in May 2005.

jur: How did you become interested in this area of research?

I've always been interested in using Computer Science to contribute something meaningful to society. Computational Biology is perfect for that because it enables me to help solve important medical problems using my skills in Computer Science.

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

After getting my B.S. I plan to get my P.H.D. in Computational Biology. Following graduate school I'd like to enter into professional research.

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

There were only a few papers written about the topic when I was conducting my research, so I found it difficult to find good test cases. I was able to contact a few professors at other universities who were very helpful in creating new test sets.

When I finished running my experiments I was left with a mountain of data, none of which was particularly meaningful. Analyzing the data and extracting information from it was the most difficult part of the project, but that made the completion the most fulfilling.

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

If you want to get into research, it helps to get an assistantship with a professor first. I worked for a year with a professor who's research interested me, and I don't think I would've been able to conduct my own research without that prior experience. I think anyone who wants to pursue research should try to get involved in a project that interests them before starting on their own work.

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Inducible Variations in Functional Connections Between M1 and Muscles

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Introduction

pike-triggered averages (SpikeTA) of rectified electromyographic activity (EMG) provide one way to examine the relationship between the firing rate of action potentials from one neuron and the activity of one muscle, recorded by EMG. By summing together multiple fragments of EMG aligned at the time of the neuron's action potential, one can evaluate the functional connection between the neuron and the muscle. If this summation produces a significant peak or trough, it may indicate that the neuron has either a facilitatory or suppressive input onto the muscle, respectively, that is timelocked to the firing rate of the neuron. If this peak or trough is determined to be significantly different from the baseline activity at a fixed time following each of the cortical neuron's action potentials, then it may represent a direct connection to the motoneuron pool.¹ Such a peak or trough is referred to as a post-spike effect (PsPE). However, if the summed EMG fragments produce no peak or trough but instead show a rather level line of noise, there is no functional connection between the neuron and muscle.

In the bulk of literature, PsPEs are believed to be constant. Only few studies have shown that the functional connections represented in PsPEs are not constant, but vary randomly



Figure 1: Figure 1A shows PsPEs present in error trials, but not in correct trials in the SpTAs of one neuron and seven different muscles. Figure 1C shows PsPEs present in correct trials, but absent in error trials in the SpTAs of one neuron with three different muscles. Significance was determined at the p <0.05 level. P values for each SpTA effect are located on the figure on the y axes.

in time while executing a motor task.² Other studies claim this PsPE variation to be dependent on the level of EMG activity.³ Unpublished experiments from our lab agree that PsPEs fluctuate, but rather than an EMG-level dependent or random variation of PsPEs in time, we found the variance to be specific to the type of movement and dependent on whether the movement was executed correctly or incorrectly.

Specifically, these unpublished findings from our lab demonstrated that PsPEs that were present during movements performed according to instruction, or correct movements, often became deactivated during incorrect movements. In fact, this observation, displayed graphically in Figure 1A, was very frequently observed. Only rarely did a neuron-muscle pair exhibit a pattern, such as that shown in Figure 1B, in which a functional connection was absent during correct trials, but became activated during error trials. Interestingly, if there was no PsPE produced by one neuron-muscle pair in all trials grouped together, a significant PsPE was often observed when only using physiological activity recorded during correct movements. Only very rarely did PsPEs emerge when only using activity recorded during error trials if there was no PsPE produced from all trials grouped together. Furthermore, PsPEs were also found to fluctuate with a specific type of finger movement. As shown in Figure 2, PsPEs that were present during one finger movement were often absent during a different finger movement, with no EMG level-dependence on this variation. Therefore, it seemed that PsPEs vary depending on whether a movement is executed according to instruction and vary by the type of movement being performed.

Variation in PsPEs would be more effectively demonstrated if one could somehow show that they can be rapidly and reproducibly changed. Therefore, we designed a task that required a monkey to operantly condition the neural activity produced by one M1 neuron to fire synchronously with the motor activity produced by EMG from one muscle. Presumably, the ability to operantly condition the synchronous firing of M1 neurons and muscles would implicate that some change in the functional connections between such neurons and the muscles must be occurring. Given the potential, rapid dissociation of neural activity from muscular activity that has been recently shown,⁴ these variations in functional connections between motor cortex and muscles seemed potentially alterable on a small time scale. However, it remained unclear how variations in PsPEs, and therefore the strength of functional connections between motor cortex and muscle fibers, would change and whether these potential changes would be reproducible if the synchronous activation of one neuron and one muscle was operantly conditioned.

The feasibility of operantly conditioning cortical neurons and muscles has been demonstrated in previous studies in which both cortical neurons and muscles have been independently operantly conditioned to increase their physiological activity. In one experiment, rats only received rewards with an increased muscular response to the H reflex.5 Since this reflex can occur in the absence of cortical input, this demonstrated the ability to operantly condition an increase in muscular activity alone, and therefore condition a specific muscle's activity. In other studies, single neurons that initially held variable relationships to specific movements became increasingly movement-specific over a period of time during which they were operantly conditioned to increase their firing rates,6 demonstrating the potential to operantly condition neural activity and reinforce the co-modulation of simultaneous neural and muscular activity.

Operantly conditioning the activity of one neuron at a time has also been complimented by experiments that operantly conditioned the activity of pools of neurons together. In rats, neural population functions from motor cortex extracted during bar-pressing behavior could replace bar-pressing behavior altogether as a reward contingency, suggesting that rewards can alter firing patterns of groups of neurons in the absence of the original muscular activity first associated with the neural population function.⁷ Therefore, we predicted there would be changes in PsPEs as the reward contingency changed from the squeeze task to the co-modulation of neural and muscular activity from one neuron-muscle pair. This would indicate a real, reproducible variation in PsPE that is task-specific and not simply a random occurrence over different time periods.

The disputed variation in the functional relationships between neural and muscular activity has also been previously suggested by experiments that reinforced the dissociation of isometric EMG activity from cortical unit activity. Monkeys were able to produce operant bursts of unit activity in the absence of isometric EMG activity,⁸ implying a necessary change in functional connectivity between motor cortex neurons and muscles. Therefore, operantly conditioning one neuron to fire synchronously with different muscles in isolated blocks of trials repeatedly seemed feasible and held the potential to demonstrate that PsPEs were not only inconstant, but could be reproducibly changed.



Figure 2: The figure above shows the variations in SpTAs of the same neuron-muscle pair during different finger movements, denoted by the column titles. The number represents the finger involved in the task while the letter "f" or "e" represents flexion or extension, respectively. For example 1f represents 1st finger flexion task, whereas 5e represents 5th finger extension task. W represented wrist. This neuron-muscle pair showed obvious PsPEs in 2f, 4f, 1e, 4e, and 5e, but during none of the other finger movements.



Figure 3: The above figure shows the raw data for a typical recording during the switch from the squeeze task (the half of the picture left of "B") to the OC task (the half of the picture right of "B"). The bottom sixteen rows represent the EMG activity of the various muscles, with each row representing a separate muscle. The row labeled A represents the raw neural activity recorded. The row above A is the discriminated pulses from the muscle being conditioned at the switch to the OC task. The row below A is the discriminated neuron pulses. At B, the task was switched to OC task. Noticeably, there is a change in the frequency of EMG bursts, of neural firing rate, and of tonicity in both.

Methods

One monkey was initially trained to perform a visuallycued squeeze task. As the monkey sat in a primate chair, the left elbow was restrained in a molded cast, and the left hand was free to grip a manipulandum consisting of a piece of PVC pipe with a flap of the same pipe removed and secured a few millimeters from the pipe by a screw, providing a cylindrical structure for the monkey to squeeze. Beneath the flap, there was a microswitch that was closed if the monkey squeezed the flap. Each monkey typically placed its thumb on the back or inside of the cylindrical manipulandum, gripping the flap with his hand. In front of the monkey, there was a display with a row of 3 LEDs.

At the beginning of each trial in the squeeze task, a yellow, centered LED was illuminated. The monkey was required to wait an initial hold period of 1500 ms before a red, LED on the far left was illuminated, signaling the monkey to squeeze the manipulandum. If the monkey squeezed as instructed, thereby closing the microswitch, a green light between the yellow and the red light was illuminated, and the yellow light was extinguished, cueing the monkey that the squeeze was occurring. The monkey had to maintain the squeeze for a final hold period of 150 msec in order to complete the trial and receive a water reward. If the monkey failed to squeeze, the trial ended in an error and a new trial began with the illumination of the yellow light, signaling the beginning of the initial hold period.

The activity of an M1 neuron was detected by a microelectrode, then discriminated on-line. The largest action potentials were used to trigger a one-shot which emitted a six-millisecond pulse. A TEMPO server then read this information into one channel and generated pulses of light in one red LED with each pulse it received from the one-shot. A separate channel carried information generated by the EMG activity of one forearm muscle. First, the EMG activity recorded from a chronic implant in one muscle was amplified and discriminated on-line. Bursts above the noise were used to trigger a one-shot which again emitted a six-millisecond pulse that the same TEMPO server read, activating pulses of light in the other red LED. Using this setup, we could provide the monkey with visual feedback about simultaneous muscular and neural activity from one muscle and one neuron.

After recording 10,000 spikes of baseline activity while the monkey executed the squeeze task, we switched him to the operant conditioning task. During the operant conditioning task, the monkey was rewarded for producing a threshold number of simultaneous pulses on both channels within a given time period. Both the threshold number of pulses and the time period were subject to our control. If the monkey accumulated a greater number of simultaneous pulses than threshold within the specified time frame, the monkey received a water reward and the pulse count in TEMPO was reset to zero. Otherwise, at the end of the time period the pulse count in TEMPO was reset to zero as the next time period began. The lights used



Figure 4: This figure represents the SpTAs from one neuron and 16 different muscles (each muscle in a different row). Each column represents the averages computed from physiological activity occurring in separate tasks. The first column represents the SpTAs from activity generated during the OC task with the ED45 muscle as the conditioned muscle, with the second column representing the OC task with the conditioned muscle being FDPU. The last column represents the SpTAs generated after returning to the OC v ED45 paradigm. Noticeably, the SpTAs showed roughly the same specific patterns in both the first and second time a specific neuron-muscle contingency was included in the OC task. Therefore, the variations in PsPEs are reproducible and seem to depend in part on the muscle being conditioned.

in the voluntary movement tasks were extinguished during OC so the monkey would be aware that rewards were being administered both red LEDs simultaneously and that the task was now the OC task.

Once a neuron was well-isolated, each recording of an M1 neuron consisted of a similar sequence of tasks that was as follows: 1) voluntary squeeze task (to record baseline SpTAs) 2) OC task 3) voluntary squeeze task (to record any changes in baseline due to the OC task). Time sequence 2, the OC task, was executed with one muscle until around 10,000 spikes accumulated. Then, the OC task usually remained on and a different muscle replaced the first muscle in the OC contingency. This meant the monkey now had to increase the synchronous activity of the same neuron and a different muscle. Each recording session typically included multiple repetitions of the OC task with different muscles after accumulating around 10,000 spikes before selecting each new muscle.

Since this is still a pilot study, the muscles involved in the contingencies were selected for different reasons each time. Sometimes, we attempted to strengthen potentially tiny effects and other times we attempted to create obviously non-existent effects. Over time, it seemed that whenever a neuron-muscle pair being conditioned in the OC task produced an obvious PsPE, it caused the activation of even more effects. So we began conditioning the same neuron-muscle pair multiple times interleaved with other muscles to see if these same patterns reappeared and returned depending on the specific muscle being conditioned rather than because of random changes in PsPEs naturally occurring over time.

Data collection

After training, aseptic surgery under isoflurane anesthesia was used to perform a craniotomy over the right central sulcus at the level of the hand representation, to implant a rectangular Lucite recording chamber over the craniotomy, and to implant head-holding posts. Once the monkey had recovered from this procedure and had become accustomed to performing the squeeze task with its head held stationary, EMG electrodes made of 32 gauge, Teflon insulated, multi-stranded stainless steel wire (Cooner AS632, Chatsworth, CA) were implanted percutaneously using aseptic technique in 8 to 16 forearm and hand muscles under Ketamine anesthesia, using techniques adapted from those of Cheney and colleagues.9 For forearm muscles, each wire was stripped of insulation for 1 mm, passed retrograde for 2 mm into a 23-gauge hypodermic needle, the needle was passed percutaneously into the muscle belly, and then the needle was withdrawn, leaving the wire tip fishhooked in the muscle belly. To implant intrinsic muscles of the hand, wires were tunneled separately for each muscle from a 5 mm incision on the dorsal aspect of the forearm to a 5 mm incision on the dorsal aspect of the hand, wire tips were stripped and inserted into the muscle belly using hypodermic needles, and then the incisions were closed with subcuticular, absorbable suture.

For both forearm and intrinsic muscles, 2 wires were placed 5-10 mm apart in the long axis of each muscle belly to provide a bipolar recording configuration. Four bipolar pairs were led to a single external connector. Appropriate location of each bipolar pair was confirmed by observing the movements evoked with intramuscular stimulation (1-sec trains of 100 Hz biphasic, constant current pulses, 200 msec per phase, 10-1000 mA).

Externally, the wires and connectors were held in place with Tegaderm, covered with a self-adhesive wrap (Vetrap, 3M), and the monkey was placed in a jacket (Alice King Chatham, Hawthorne, CA) with heavy Cordura sleeves to prevent removal of the electrodes. During subsequent recording sessions the sleeve and self-adhesive wrap were removed, exposing the connectors held in Tegaderm. Implanted electrodes typically functioned well for 2-3 weeks, after which they were removed by loosening the elastic adhesive tape with acetone and then gently pulling each wire out through the skin. Then, another set of EMG electrodes was implanted. Muscles implanted typically included 16 of the following: thenar eminence (Thenar); first dorsal interosseus (FDI); hypothenar eminence (Hypoth); flexor digitorum profundus, radial region (FDPr); flexor digitorum profundus, ulnar region (FDPu); flexor digitorum profundus, proximal ulnar region (FDPpu); flexor digitorum superficialis (FDS); flexor carpi radialis (FCR); palmaris longus (PL); flexor carpi ulnaris (FCU); abductor pollicis longus (APL); extensor pollicis longus (EPL); extensor digiti secundi et tertii (ED23); extensor digitorum communis (EDC); extensor digiti quarti et quinti (ED45); extensor carpi radialis (ECR); extensor carpi ulnaris (ECU), and supinator (Sup).

Thereafter, in daily recording sessions, conventional techniques were used to record single M1 neurons simultaneously with EMG activity from the implanted forearm and hand muscles (EMG amplification 2,000-100,000 x, bandpass 0.3-3 kHz, sampling frequency -4 kHz per channel) as the monkey



Figure 5: Represents the SpTAs generated from one neuron and 16 different muscles (each row is a different muscle). Each column represents a different task. For example, OC v ECRB represents the OC task with the ECRB muscle as the conditioned muscle. Each OC task was completed twice, with the second time designated with a 2 at the end of the task name. "Post" represents a return to the squeeze task.

performed the task. One data acquisition interface was used to store data to disk on one host PC, which also provided a scrolling display of all neuron and EMG recordings (Power1401 interface, Spike2 software, Cambridge Electronic Design, UK). A second identical data acquisition interface and host PC running AVE software (courtesy Shupe, Fetz and Cheney) were used concurrently to form initial on-line averages of rectified EMG for each channel using data segments extending \pm 50 msec from the time of all neuron spikes. M1 neurons that produced an effect evident in the on-line average of at least one muscle were recorded until 10,000 spikes had accumulated before switching the task. Each recording lasted as long as satisfactory isolation could be maintained or until recording 10,000 spikes while the monkey executed the squeeze task one final time at the end of the task sequence.

Analysis of SpikeTA effects

If any SpTA showed a peak (or trough) still significant at the p < 0.05 level, the neuron was accepted as producing a PsPE. The variability in PsPEs across different tasks was evaluated in terms of significance level changes and by changes in the amplitude of the PsPE, measured the peak percent increase (PPI). The PPI was calculated by finding the maximum (for a peak, or minimum for a trough) of the SpTA waveform between the onset and offset, subtracting the baseline mean, and then dividing the result by the baseline mean and multiplying by 100.

Results

The monkey quickly understood the switch from the voluntary task to the OC task without any trouble, evident



Figure 5B: Plots of the PPIs for two different neuron-muscle pairs taken from Fig. 5A. The top line represents the PPI changes across the tasks in ECRB. The bottom line represents the PPI changes across the tasks in FCU. Evidently, PPI varies with the task paradigm, and similar PPIs are observed when the same OC task is repeated. Therefore, changes in PPI are not simply randomly occurring with time.

in the rapid change in both neural and muscular activity in response to the task change. A typical example of the physiological changes that accompanied a switch from the squeeze task to OC task is depicted below in Figure 3. The switch from squeeze task to OC task was immediately followed by the monkey releasing the squeeze manipulandum and the initiation of small wrist and hand movements that persisted until the voluntary task began again and he resumed his grip of the manipulandum. This change in behavior was reflected in the patterns of EMG activity in each muscle, with more tonic activation of some muscles, and rapid bursts of much greater frequency in others. The rapid changes in behavior and muscular activity were accompanied by a similarly sudden increase in the neuron's firing rate. Often a more tonic neural discharge occurred as well.

When switching between muscles the monkey was required to activate in the OC task, noticeable changes in SpTAs were observed, with PsPEs of one neuron and many muscles changing depending on the task paradigm (OC or voluntary squeeze tasks), and the muscle being conditioned. In 59 neuron-muscle pairs, switching from baseline to OC task, from an OC task with one conditioned muscle to an OC task with a different conditioned muscle, and from the OC task to baseline again, the significance levels and the shapes of the PsPEs changed. Remarkably, as evident in Figure 4 below, when re-conditioning a previously conditioned muscle during the same recording session for a given neuron, SpTA patterns in all muscles re-emerged in remarkably similar fashion each time the specific muscle was operantly conditioned. Similarly, each time the monkey began the squeeze task again, either before or after the OC task, the SpTA patterns for the same neuron across all muscles regained their original forms.

Interestingly, even when a neuron-muscle pair's PsPE did not vary in significance level from one experimental task to the next, the height of the SpTAs, measured by peak percent increase (PPI), often changed, indicating a task-dependent variation in PsPE amplitude. As depicted in Figure 5 below, the height of the SpTAs varied with the task paradigm in both neuron-muscle pairs with facilitory (positive PPI) effects and inhibitory (negative PPI) effects. Like the variations in significance level, each time the same neuron-muscle pair was conditioned, the PPIs across multiple muscles returned to about the same number. However, in this preliminary study, the number of neuron-muscle pairs that regained the same significance level and PPI each time they were operantly conditioned with specific muscles has not yet been quantified.

In all, SpTAs were obtained for 50 neurons and 176 neuron-muscle pairs in the M1 hand representation of the monkey. However, in this preliminary report, only 12 neurons were used for the analysis. To quantify the amount of neuron-muscle pairs that changed in significance level and in PPI across a population of neuron-muscle pairs, the following anlaysis was performed. If the PsPE was significant during at least one of the OC tasks, the PPI occurring during this OC task was extracted. Then, for each neuron-muscle pair, all extracted PPIs were compared to find the largest PPI change from baseline to OC task. The maximum change in PPI from baseline to the OC task for each neuron-muscle pair was compiled into a histogram to observe the frequency of various amounts of change in PPI over baseline during the OC task.

The histogram, shown in Figure 6 below, showed that 117 neuron-muscle pairs produced no significant change in PPI when going from baseline to the OC task, whereas 59 neuron-muscle pairs did produce effects during the OC task and had PPIs that varied from baseline by as much as 20 in both positive and negative directions. Furthermore, PsPEs induced during the OC task were mostly facilitory rather than inhibitory.

On rare occasions when two neurons were being recorded on the same electrode, the monkey was able to increase the firing rate of one unit without largely affecting the isolation or firing rate of the other unit. More interestingly, the SpTA changes in one neuron produced during the OC task were not mirrored in the SpTA changes of the other neuron. In most all neuron-muscle pairs, SpTAs recorded in the return to the squeeze task after the OC task was finished strongly resembled the SpTAs recorded during baseline, implying the induced changes in functional connections were task-specific and not permanent.

Discussion

Evidently, changes in functional connections can be influenced by the voluntary synchronous activation of a neuron-muscle pair. However, it is unclear whether these changes result from an increased rate of tonic neural and EMG activity or an increase in total physiological activity from cortex and all muscles that is causing these changes in SpTAs. It is clear that when the task switches from the squeeze task to the OC task, the monkey usually increases the firing rate and/or tonicity of the neuron and/or muscles. However, the changes in functional connections that occurred in response to task changes were very different across all sixteen muscles, and differed from neuron to neuron as well. Therefore, it is reasonable to conclude that it is not merely the increased tonicity and activity of both neurons and muscles during the OC task that was causing the observed changes in SpTAs, but the actual changes in functional connections between the neuron and the muscles being recorded.

Interestingly, the neuron-muscle pair being conditioned to synchronously fire did not always produce a PsPE in the OC task, even when inducing effects in other muscles. This is expected because not every neuron recorded is presumed to have a functional connection with one of the sixteen muscles we were recording. Therefore, increasing the simultaneous activity of both neuron and muscle can not activate a connection that is not present. However, the fact that SpTA changes could be induced in many muscles by conditioning the neuron with a different muscle implies that the monkey was making some movement with his hand that increased the synchronous activity of the neuron and the required muscle, while activating functional connections between that neuron and other muscles. Again, this supports the idea that it is not merely the increased firing rate or tonicity of the neural and muscular activity that is responsible for the observed changes in SpTAs, as the monkey is increasing the EMG burst frequency and tonicity across all muscles, with only some producing changes in SpTAs.

The reemergence of extremely similar SpTA patterns across all muscles when conditioning the same muscle multiple times in separate blocks during one recording provides evidence that the SpTA changes are a real, task-specific phenomenon, not just random fluctuations in functional connections that occur



Figure 6: The above graph depicts a histogram of the number of neuron-muscle pairs during the OC task with specific change in PPIs over baseline. As evident by the graph, 117 neuron-muscle pairs did not have a significant change in PPI during the OC task over baseline and were therefore plotted as 0. However, a large number of neuron-muscle pairs did have a significant change in PPI during the OC task, with as high as 20 and as low as -20 change in PPI over baseline.

over time. Based on our unpublished findings that PsPEs vary with the type of finger movement being executed, this finding is not surprising because the monkey is presumably executing a different task each time a different muscle is added to the OC contingency. Thus, it is not surprising that the PsPEs were often specific to the OC task neuron-muscle pair being conditioned and could be reliably reproduced by repeating a the OC task with the same neuron-muscle contingency.

In the future, the number of neuron-muscle pairs that regained the same significance level and PPI each time a specific neuron-muscle pair was operantly conditioned to be synchronously activated should be quantified to ensure that this change is, in fact, real. In addition, the increased firing rate and muscle activity seen during the OC task must also be controlled for to ensure it is not causing the observed changes in PsPEs. One method of doing this would be to plot the correlation between changes in the firing rate of the neuron against changes in PPI and significance levels observed in each separate task. This should be done for each neuron so the population of neurons can be evaluated. Furthermore, while it would also be useful to quantify the correlation between changes in EMG activity for each muscle with changes in PPI and significance levels to confirm that changes in PsPEs are not due to increased EMG activity sine other studies have claimed EMG-dependent variation in PsPEs.³

In future studies stemming from this project, it would be interesting to include a second neuron in the OC paradigm to determine whether the changes in PsPEs observed could be induced and constantly reproduced in two neurons simultaneously, since the changes in PsPEs recorded from one neuron did not necessarily alter the SpTAs produced by neurons recorded simultaneously on the same electrode. In addition, it would be interesting to attempt to execute this experiment in the motor cortex ipsilateral to the arm being moved. Lastly, I would like to include the suppression of specific muscles into the OC task contingency so that the monkey can not simply contract all muscles to get rewarded, but has to selectively activate only one or a few muscles.

However, before these studies could begin, we must first complete this study by recording more data and quantifying other aforementioned changes in SpTAs that seem to be occurring between the squeeze task and the various OC tasks with different muscles. For example, are neurons more often losing effects or gaining effects during the OC task? Are the effects ever changing from inhibitory or facilitory? If not, could we induce this? Furthermore, how often was a facilitory effect induced in one muscle during the OC task accompanied by an inhibitory effect in an antagonistic muscle? As mentioned, it will also be necessary to control for increased neural firing rate, muscular activity to ensure that these are not responsible for the observed changes in SpTAs during the OC task. In addition, it would be ideal to measure the simultaneous activity in other muscles not being recorded elsewhere in the body to ensure that increased tension across other muscles in the body are not simply heightening the number of EPSPs in the recorded muscles, making the increased neural firing rate during the OC task the little spark that activates a functioncal connection between the neuron and the muscle.

Conclusion

PsPEs are variable and can be induced and reproduced when operantly conditioning the synchronous activity of one M1 neuron and one muscle. Switching between the voluntary task and the OC paradigm produced noticeable changes

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in SpTA effects with three principle constant findings: 1) SpTAs of one neuron and many muscles vary in significance and PPI depending on the task paradigm (OC or voluntary squeeze tasks) 2) conditioning different muscles produces different SpTA patterns in the same neuron-muscle pairs and 3) conditioning one neuron-muscle induces similar SpTA patterns across all muscles each time it is added to the OC paradigm.

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About the Author

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

To truly understand how humans control our muscles in everday life, we must understand the nature of the functional connections between the brain and the muscles. In the long term, such understanding will allow us to construct more effective brain-machine interfaces, robotic limbs that are directly driven by signals extracted from motor cortex.

jur: What motivated you to do this project?

Wondering how our brains control such a variety of movements so rapidly and with such precision in such a variety of contexts has always been intriguing to me. I really wanted to understand how the brain was forming such communications between the brain and muscles and whether these connections were changing frequently or relatively stable. Understanding this would provide insight into how we can more effectively help others with trouble controlling their muscles.

jur: What was the biggest obstacle of this project and how did you overcome it?

The biggest obstacle of this project involved the use of macaques, because if they did not feel like working one day, you would lose a whole day of recording. Our experiments were often subject to their moods and/or temperament which could be frustrating at times. However, with time, the macaques learned to cooperate much better for rewards.

jur: Any advice you can give to undergrads who would like to undertake research like this (or research in general)?

Follow your interests and find a mentor who will 1) take the time to explain things to you, 2) help you develop your interests by knowing what has been done/should be done and be able to reference you to papers that interest you, and 3) is openminded and likes student involvement in the lab. If something interests you, try to get involved in related research. Chances are, if you are interestd in a research project enough, it will be a positive experience if you have the right mentor who will invest time in you and who likes to teach you and be involved in the lab.

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Aberration Correction Using Adaptive Optics in an Optical Trap

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Introduction

The processing of optical trapping is extremely useful in the biological sciences for manipulating microscopic particles and organisms using laser light. Imperfections in the elements comprising the optical trap cause deformations in the laser beam, which decrease the effectiveness of the trap. The wavefront distortion on the beam can be described by Zernike polynomials or disk harmonic functions. The coefficients of these functions can be manipulated to compensate for the aberration making a diffraction limited spot, and therefore, a more effective trap. The aberration correction is performed using an adaptive simulation in the programming language MATLAB. Three elements are required in an adaptive optics system: a wavefront sensor, a corrective element, and a feedback or control loop. A sensor consisting of a pinhole and a powermeter, or a camera and a cost function are used to detect and measure the aberration modes. A means for correcting the aberrations detected by the sensor was created using a spatial light modulator (SLM). The adaptive control scheme for adjusting the Zernike coefficients or disk harmonic functions involves testing two steping algorithms. Simulating the adaptive optics system, a program was written to correct for a single mode. Multiple modes were adapted in an iterative scheme and movies were made of the simulation. The system was able to correct for this aberration, bringing the laser to a tighter, diffraction-limited spot. This paper describes the simulation of aberration correction in an optical trap using adaptive optics.

Optical Trapping

Optical trapping is a process by which microscopic particles, organisms, or parts of organisms are manipulated by optical forces. When the laser beam hits the small particle, the individual rays of light are bent due to reflection and refraction, and the change in the momentum of the light creates a force on the particle that sums to a net force. This force can be resolved into two components: a scattering force acting in the direction of the propagating laser beam and a gradient force that pulls the particle toward the axis of the beam, as shown in Figure 1. Provided the index of refraction of the particle is greater than the index of refraction of the surrounding medium, the particle will be pulled to the beam.¹ A single beam optical trap is created when the gradient forces are greater than the scattering forces.

The optical trapping system consists of a laser propagating through four lenses and into an inverted microscope in order to trap a specimen on the stage. Here the beam partially overfills the microscope objective. Moving from the laser to the objective, the focal length of the lenses are: 500mm, (SLM, see below), 750mm, 300mm, and 150mm. The microscope objective is 100x (focal length of 1.64mm). When constructing a single beam optical trap, an objective with a high numerical aperture is necessary in order to bring the beam to a tight focus. Two telescopes, created by L1 and L2, and L3 and L4 (Figure 2), are used to match the size of the beam at the entrance pupil of the objective to the spatial light modulator (SLM).² A spatial light modulator is capable of converting data in incoherent optical form into spatially modulated coherent optical signals. There are two types of SLM's: electrically written and optically written. The latter is used here and will convert an incoherent image into a coherent image for further processing by the rest of optical trapping elements. The SLM can also provide image amplification and wavelength conversion. It creates spatial filters that can be modified in real time.³ In our system, the SLM is used to correct for aberrations in the optical trap by modifying the phase of the wavefront of the laser beam.

A Quadrant Photodiode (QPD) is a position sensing device that contains four sensing areas or quadrants. The QPD senses



Figure 1. Forces on trapped particle



Figure 2. Experimental set-up for a QPD Optical Trap

the position of the laser beam on the sensing quadrants. The experimental set-up for a QPD optical trap is shown in Figure 2. An adaptive optical system is created and used to correct for the aberrations in the trap. A pinhole replaces the camera and eliminates any extraneous rays. On the backside of the pinhole is a powermeter.

Trapping Efficiency

The force needed to trap a particle in the optical trap is known as the trapping force, F, and is proportional to the power of the laser, P, the speed of light in a vacuum, c, and the index of refraction of the particle, n. The trapping force also depends on a factor Q.

$$F = \frac{nP}{c}Q$$

The force required to remove a particle from the optical trap can be calculated from the Stokes drag force, F_{Stokes} .

$$F_{Stokes} = 6\pi \eta v$$

Where r is the radius of the particle, v is the velocity, and η is the viscosity of the fluid in which the particle is suspended. A second force, F_{light} , known as the escape force, arises from the momentum flow within a laser beam.

$$F_{light} = \frac{nP}{c}$$

The trapping efficiency can be calculated by taking the ratio of the Stokes drag force to the escape force.⁴ Typically the trapping efficiency of the optical trap is between 5% and 15%.

Aberrations

Aberrations in the laser beam create a less efficient optical trap. The trap contains many elements, none of which are perfect. Real lenses neither have perfect curvature nor are they perfectly smooth. These deformations cause aberrations in the beam. After traveling through a number of optical elements, the beam can become severly aberrated. Some of the rays will deviate slightly, scattering light in different directions and creating an unfocused spot. This distortion is not beneficial for optical traps because it indicates a large amount of diffraction, which makes the trap less effective.

In order to simulate the aberration correction in the trap a few functions are generated in MATLAB. A pupil function is created, P(x,y), with a radius of 1. In an experiemental setup, the pupil function would be used to eliminate any extraneous rays of light by only allowing light to pass through a small hole, or pupil. In the diffraction limited case, the Fourier Transform of this pupil function creates a point spread function in the form of an Airy Disk. Figure 3 shows what the point spread function would look like if no aberration is present (an Airy Disk).

Some random wave distortion is generated to simulate the aberration. This wave distortion is described by the function $W_d(x,y)$. The field at the pupil can be represented by the following equation, where the wave number, $k = 2\pi/\lambda$.

$U(x, y) = P(x, y) \exp(jkW(x, y))$

The known wavefront W(x,y) represents what is displayed on the spatial light modulator. What reflects off of the SLM is the field at the pupil, U(x,y). U(x,y) is defined below as the pupil function multipled by a phase term for the known wavefront and a phase term for the generated wavefront distortion.

$U(x, y) = U_d(x, y) \exp(jkW(x, y))$

The Fourier Transform of this function then gives the point spread function.

Wavefront distortion can be described mathematically by a series of functions. For optical systems, Zernike polynomials are a convenient basis for this expansion. Interpreting these polynomials provides a description of the aberrations present on the wavefront of the laser beam. In this research, MATLAB software was developed to describe and simulate the wavefront distortion of the laser beam. Aberrations in the wavefront are described by the sum of the Zernike modes (Z_i) present multiplied by a Zernike coefficient (a.).

$$W(x,y) = \sum a_i Z_i(x,y)$$



Figure 3. Airy Disk

This equation describes the added optical path length taken by the rays as a result of the aberrations. The added phase due to the aberrations is described by a complex term which, when multiplied by the pupil function, gives a generalized pupil function. The Fourier Transform of this generalized pupil function is the real aberrated point spread function given in Figure 5. The intensity of the beam is the squared magnitude of the point spread function. Once the aberrations are clearly described, the Zernike polynomials can be changed to compensate for them.

Zernike Polynomials

Zernike polynomials are used to describe the wavefront distortion on the laser beam. The polynomials are used to create both the known and distortion wavefronts during the MATLAB simulation. For Zernike polynomials, the mode number is defined as

$$j = \frac{n(n+2) + m}{2}$$

where n is the radial order

$$n = roundup \left[\frac{-3 + \sqrt{9 + 8j}}{2} \right]$$

and m is the angular frequency

$$m = 2j - n(n+2)$$

Using the radial order and angular frequency, any Zernike mode can be indexed. Figure 4 shows the first fourteen modes as intensity plots. The first order modes are xy-tilt. The second order modes are astigmatism and defocus. Coma and trefoil in the x and y directions compose the third order aberrations. Coma scatters the light more in one direction than the other, and the trefoil has three peaks and troughs around the edge. Secondary coma, tetrafoil and spherical aberration make up the fourth order Zernike modes. Figure 5 is an intensity plot of a wavefront with tweleve Zernike modes present.

Disk Harmonic Functions

An alternate set of functions known as the disk harmonic functions may be used instead of the Zernike polynomials and are described below.



Figure 5. Aberrated beam created with Zernike polynomials

$d_{nm}(r,\theta) = J_m(2\pi l_{nm})\exp(im\theta)$

where J_m is the mth-order Bessel function of the first kind, $n = 0, 1, 2, ..., m = 0, 1, \pm 2, ... and l_{nm} = 0 \Rightarrow m = 0 and is$ given by

$$2\pi \int_{0} J_m(2\pi l_{nm}r) J_m(2\pi l_{\nu m}r) r dr = a_{nm} \delta_{n\nu}$$

where a_{nm} is the normalization constant and d_{nv} is the Kronecker delta ($d_{nv} = 0$ for $v \neq n$, $d_{nv} = 1$). An intensity plot of a disk harmonic function is shown in Figure 6 as an example. The Zernike polynomials are replaced with the disk harmonic functions and the two simulations are compared. The disk harmonic functions spread the light more evenly in disks around the center of the beam, this can be seen in Figure 7. However, both functions are capable of being adapted to correct for this aberration. This research aims to correct for these aberrations using adaptive optics techniques so that the ray bundle focuses to a diffraction limited spot, providing an overall sharper focus and more effective trap.

Adaptive Optics

Adaptive optical systems are comprised of three parts: a sensor, a corrective element, and a feedback or control loop.



Figure 6. Disk Harmonic Function



Figure 7. Aberrated beam created with disk harmonic functions

First, a sensor needs to be developed in order to detect and measure the aberration modes. Next, a means for correcting the aberrations detected by the sensor will be created using a spatial light modulator. Finally, an adaptive control scheme for adjusting the Zernike coefficients or disk harmonic functions will be developed to minimize the effects of aberrations making a diffraction limited spot, and therefore, a more effective optical trap.

Cost Sensor

The sensor is created with one of two set-ups: a pinhole and a powermeter, or a camera and a cost function. The pinhole and powermeter can easily be inserted into the optical trapping system after the laser reaches the microscope. For the purposes of simulation, a pinhole function is created and multiplied by the intensity. Integrating over the area gives a value, J, the cost of the aberration. Inserting a camera into the optical trap allows a number of other cost functions to be tested. The following cost functions were used during simulation, where m = 2, 3, or 4.

$$J = \sum \sum I_{ij}^{m}$$
$$J = \sum \sum I_{ij} \log I_{ij}$$
$$J = \sum \sum r_{ij}^{2} I_{ij}$$

A liquid crystal spatial light modulator is the actuator used to correct for the aberrations in the optical trap. It does this by adjusting the phase of the wavefront. Software is developed to aid in modeling the wavefront sensing and adaptive control. The active spatial light modulator optical elements will correct for the aberrations in the optical trap given the information from the wavefront sensor.

Adaptive Algorithm

An adaptive control scheme is generated to correct for aberrations in the laser beam one mode at a time. A stepping algorithm is used to maximize the cost function, which indicates a maximum intensity characteristic of a focused beam. For the Zernike polynomial adaptation, the surface of the cost function plotted against two of the Zernike coefficients can be thought of as a bowl. If the initial guess for the value of the Zernike coefficients is on the edge of the bowl, steps are taken toward the center by adjusting one Zernike coefficient, then another, and repeating this process iteratively. The steps taken depend on the gradient of the cost function, which can be calculated using the current and previous values of J and a as shown below.

$$\nabla J = \frac{J_n - J_{n-1}}{a_n - a_{n-1}}$$

The following algorithms are tested to find the value of the Zernike coefficient, a, that best corrects for the aberrated mode, a_d . The first one calculates the next guess for the Zernike coefficient using the previous guess, the sign of the gradient, and a constant step size. The second is based on the the previous Zernike coefficient, the gradient, and a step size.

$$a_{n+1} = a_n + sign(\nabla J)\mu$$
$$a_{n+1} = a_n + \mu \nabla J$$

The purpose of this research is to correct for aberrations in an optical trap using adaptive optics. After the aberration modes present on the wavefront of the laser beam are known, the spatial light modulator can be used to compensate for the aberrations. Adjusting the Zernike coefficients will minimize the aberrations and cause the laser to focus to a diffraction limited spot, thus increasing the effectiveness of the optical trap.

Methods and Procedures

MATLAB code was first written to calculate the Zernike polynomial for any mode; it was called in the main function to calculate all of the modes present on the beam.

It was first helpful to see how the cost function changes as a function of the Zernike coefficient. Two wavefronts were created: the distortion wavefront, which is based on the Zernike coefficient, and a wavefront that will be adapted to correct for the distortion. These two wavefronts were added together and the generalized pupil function, the point spread function, and the intensity were created. To test the different cost functions, each was plotted against the Zernike coefficient.

The main program is an adaptive system that corrects for the aberration in the beam one mode at a time. After the cost function was calculated, the algorithm was implemented. Each mode was corrected in turn and the process repeated until a desired level of performance was reached. If the aberration was not completely corrected after looping through all of the modes, the program could cycle through as many times as needed. Movies of the adaptation process were made.

The final step in the simulation of the adaptive optical system was to use disk harmonic functions to create the aberration and correct for it in place of the Zernike polynomials. This change was minor; a new program was written to calculate the disk harmonic functions and called from the main program.

Results and Discussion

The simulation began with a program that calculates the Zernike polynomial for any mode. Figure 8 is an output of this code for astigmatism, Zernike mode number 5. It shows the Zernike mode as surface and contour plots along with the



Figure 8. Zernike mode 5: (a) surface plot, (b) contour plot, (c) Zernike mode times the pupil function, (d) point spread function

mode after it is multiplied by the pupil function and the point spread function.

Next, plots of the different costs as functions of the Zernike coefficient were created. When any cost function is plotted with no aberration, it peaks at zero. If the aberration were equal to 0.1 then the peak would shift left to -0.1, indicating the value of the Zernike coefficient needed to correct for it. Three of the cost functions were calculated using I^m, as m was increased from 2 to 4, the peak gets thinner and the maximum value of the cost is an order of magnitude greater each time. A plot of the intensity times the pinhole function cost is shown in Figure 9.

The development of adaptive optics simulation software progressed in stages. First, the simulation was written to correct for a single Zernike mode. The initial guess of the Zernike coefficient was set at zero and the program calculates the cost for each new guess using the algorithms previously mentioned. A new value of the coefficient was calculated and then used to calculate the next value of the cost. This was repeated for a large number of iterations and eventually both the coefficient and the cost converged. Plots of the cost and aberration correction show the response of the adaptive optical system. The algorithms were performed using each of the cost functions and produce very similar results. It was discovered that the algorithm using a constant step size was the most efficient. A movie was created of the intensity as the coefficient was changed to compensate for the aberration.



Multiple modes can be adapted in an iterative scheme. Figures 10 and 11 show the values of the Zernike coefficient and the cost function as they were adapted to correct for an aberration of 0.1 in Zernike modes three through fourteen. In the cost function plot, each hump represents a different mode converging. The cost function being implemented here is the integral over the area of the Intensity times a pinhole function (radius of 0.1 units). The other costs were also tested and the responses look similar. Figure 10 shows the values of the Zernike coefficients, all starting at zero, as they were adapted to correct for the aberration. All of the values should theoretically converge to -0.1, since the aberration is set at 0.1 for all of the Zernike modes. According to the plot, however, two of the modes, three and five, start off converging in a positive direction, which actually makes the aberration worse. This was corrected by cycling through the modes again. All of the other modes converge in a negative direction, although none actually reach 0.1 during the first cycle. This may occur because when these smaller order modes were first being corrected, the higher order modes were still present. The first time through the system the smaller order modes may go in the wrong direction, or not go far enough, to compensate for the higher ones. Once the higher modes had been corrected during the second cycle, the lower modes began to adjust accordingly. Once one mode was corrected its new value for the Zernike coefficient was held constant. The horizontal lines show the final value for each coefficient.

The second cycle further corrects the aberration; these plots were similar to Figures 10 and 11. In the second cycle, the two values of the coefficient, three and five, that were converging in a positive direction switched to negative. All of the values



Figure 10. Cost as a function of the total iterations for the first cycle (q = 1).





start where they left off after the first cycle. Most of the other values still converge to a negative number and get closer to 0.1; if the process is continued for more cycles, an improvement in the cost would be seen each time. Also the values of the Zernike coefficients will become closer and closer to -0.1. After interpreting multiple mode graphs and viewing the movies for each of the different cost functions, it was found that the best cost functions were the radius squared times the intensity and the pinhole function times the intensity.

The disk harmonic functions are implemented in the main program instead of the Zernike polynomials. The disk harmonic functions, rather than the Zernike polynomials, are implemented in the main program. Multiple mode correction plots of the cost and Zernike coefficients are similar to Figures 10 and 11. Finally, the aberration was created using disk harmonic functions and corrected for using Zernike polynomials. In this situation the cost inreases more quickly.

Summary and Conclusion

Optical trapping is a process by which microscopic specimens are manipulated by optical forces. The physical elements in the trap cause distortions in the laser beam as it propagates through the system. It is beneficial to reduce this distortion, therefore reducing diffraction effects and creating a more effective trap. The wavefront distortion on the beam can be described mathematically by Zernike polynomials or disk harmonic functions. An adaptive optics system was created consisting of a cost sensor, a liquid crystal spatial light modulator, and an adaptive control scheme. Several cost functions were tested throughout the experiment and it was discovered that the best functions were the radius squared times the intensity and the pinhole function times the intensity. The spatial light modulator compensates for the aberration by adjusting the phase of the wavefront. The adaptive control scheme for adjusting the Zernike coefficients or disk harmonic functions involved testing two stepping algorithms. The algorithm using a constant step size produced the best results.

Simulating the adaptive optics system, a MATLAB program was first written to correct for a single mode. Multiple modes were adapted in an iterative scheme and movies were made of the simulation. The system was able to correct for this aberration, bringing the laser to a tighter, diffraction limited spot, thus increasing the effectivness of the optical trap in simulation.

The most important short term benefit of this research is that this aberration correction can now be tested experimentally on the trap. A known aberration will be applied to the laser beam and then corrected for using the code described above and interfaced through LabView. Future research involving the optical trap may lead to its use in laser eye surgery. The trap could potentially be helpful for vision correction. Before the output laser beam can be applied to a human eye it must be virtually aberration free. Any extraneous rays could severly damage the eye, and the intensity of the beam needs to be extremely concentrated in order to produce the accuracy required when working with the eye. Once the aberrations are corrected, the optical trap can be used to trap or pull on molecules or particles in the eye in order to improve vision.

Acknowledgments

• Dr. Rob Clark, Senior Associate Dean and Director (CBIMMS)

• Dr. Daniel Cole, Research Assistant Professor, Mechanical Engineering and Material Science

• Scott Kennedy, Graduate Student, Mechanical Engineering and Material Science

• Kurt Wulff, Graduate Student, Mechanical Engineering and Material Science

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About the Author

Carrie Voycheck, a senior majoring in biomedical engineering with a concentration in optics, did her research as part of the Research Experience in Physics & Astronomy for Undergraduates (REU) summer program under the guidance of Dr. Daniel Cole at the Duke University. Carrie plans to pursue graduate studies in biomedical engineering after graduation.

jur: What is aberration correction all about and what applications does it have?

The basic principle behind my project is optical trapping, a process by which microscopic particles, organisms, or parts of organisms are manipulated by optical forces. These optical forces are created on the sample by laser light. After traveling through an optical system the laser beam contains some aberration which decreases the effectivity of the trap. My project was to simmulate the correction of these aberrations in MATLAB. In doing this the laser beam will become more focused and the trap more effective. After the aberrations have been corrected the trap may be used in laser eye surgery. The processing of optical trapping is also extremely useful in the biological sciences for manipulating microscopic particles and organisms using laser light. Cellular organells and DNA can be pushed, pulled, and manpulated for a variety of purposes.

jur: What motivated you to do this research project?

I applied to a number of REU programs last summer because I want to go to graduate school starting fall, 2006, and work toward a Ph.D. in Biomedical Engineering. Ultimately I chose the REU program at Duke University because it is one of the schools I have applied to this fall. I would like to research a combination of biomechanics and optics and was placed on this project because of my background and interest in these areas. Working in the Center for Biologically Inspired Materials and Material Systems at the Pratt School of Engineering I worked in the Mechanical Engineering Department doing optics so this was a perfect fit.

jur: Any advice you could give to fellow undergrads who would like to undertake similar research (or any research in general)?

It was a wonderful experience and I had a great time. It's a really good idea if you are unsure whether or not you want to have a career in research. It allows you to test the waters and see what it is going to be like before you get to graduate school. I also met a lot of great people and more importantly made connections. Doing research at another university really helps if you are applying to graduate school, especially if you want to go to that university because people there already know what you can do. I would recommend an REU Program to anyone who is considering a career in research or attending graduate school.

The Future of Broadband Over Power Line

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I. INTRODUCTION

POR quite some time, the feasibility of transmitting communications signals over the power line, known as power line communications (PLC), has been questionable. Investigations on PLC have waxed and waned over the years, but in the last five to ten years, the advent of the Internet has prompted renewed interest. There is now a need for easily accessible, high-speed broadband communication. Because of the existing infrastructure, the power line is being considered as a possible communication channel. This subset of PLC is known as "broadband over power line," or BPL.

It is important to understand the distinction between PLC and BPL, as they are too often used interchangeably. Put simply, BPL is a specific type of PLC. For clarity, the term PLC will be used to refer to any communications scheme that is sent over the power line, regardless of bit-rate or frequency. This definition for PLC also encompasses BPL—a term which will be used to specifically designate high frequency, high bit-rate communications over the power line, such as internet access. Because BPL is a type of PLC, to examine the feasibility of BPL, one must first examine PLC as a whole. So, while the historic applications of PLC will be discussed, it serves as more of background as to what technologies lead up to BPL.

The concept of PLC is certainly noteworthy. The power infrastructure already exists nearly everywhere, eliminating the need for new lines. Such everyday unmanned devices as traffic signals, well-pumps, street lights, lighthouses, and subway cars could have the capability to be controlled over an existing network. There is still plenty of bandwidth available on the lines to support this activity.

Within the power distribution network, there are three types of distribution lines: high voltage, medium voltage, and low voltage. Running with continuous voltages from 1 kV to in excess of 100 kV and covering long distances, the high and medium voltage lines are primarily used as ties between different grid sections. Currently, these lines are paralleled by a fiber optic signaling network that has plenty of extra bandwidth for BPL.¹

On the low voltage network, at voltages less than 1 kV, the widespread distribution of the electric grid becomes an inherent advantage. Although noise and signal attenuation are problems on these networks, they still hold the potential

to carry a power line-based local area network (LAN). If these problems can be properly modeled and overcome, then there is no question that transmitting BPL is a possibility. Thus, the primary focus of this article will be on the low voltage network.

II. PLC APPLICATIONS

While the applications for PLC are practically endless, there are several that have been proven to provide the most cost effective utility: load management, automatic metering, and BPL.

Load management is a technique employed by the power company to maintain a stable grid. For a distribution system to function, the load must be matched by the generation. Currently, large kilowatt generators must adjust instantly to shifts in load. However, load management could be used to instead redirect current over different transmission lines and to different grid sections. A signal can be sent over the power line to a switching station or capacitor bank to make required adjustments that would otherwise require a secondary form of communication. This improves the overall grid stability and reduces operating costs.

Automatic metering is another practical PLC application. Rather than having an employee manually go from house to house and business to business reading the electric meter, the meter sends a signal back to a receiver. Its unique identification number and current usage to date is broadcast and consequently recorded. Although cost to the power company is reduced significantly, the communication speed so far has proven quite slow. In one application in the United Kingdom, the data transmission ran as slow as 1 bit/sec.¹

The main application of interest is BPL. With a high rate of data transmission, the power line could provide internet access, voice over IP, and other broadband services. This is beneficial to the consumer because the common power outlet becomes a gateway to the Internet and a home LAN with no need for additional wiring. The power company then has the advantage of opening up a competitive service in a market where it previously had no interest.

III. HISTORY

The idea of power line communications is not new.

Experiments on PLC began in the early days of electrical distribution. Then, the focus was on telegraph and telephone communications. Because of the lack of necessary technology, these early methods failed.

In the late 1970s, research was concentrated on automatic meter reading and load control. High-speed transmission could be obtained over short distances. However, only low-speed transmission could be attained over long distances.

One of the first PLC systems to appear was a metering system created in 1972 by General Electric. It operated in the range of 35 to 40 kHz and, at these frequencies, was not susceptible to destructive harmonics of the mains. However, losses from propagation and distribution transformer couplings proved to be a substantial barrier.²

Later, in the early 1980s, an improved metering system was developed named AMRAC III. It was first implemented by Virginia Power in the Williamsburg area in the early 1980s. The frequency of this next generation device was shifted down to the 5-10 kHz range. Here, the propagation and coupling losses were much more acceptable. Unfortunately, the interference due to harmonics at this frequency was a hindrance.²

By the late '80s, yet another system, known as the Rockwell System, was developed. In addition to metering, this system provided for load management and distribution automation. While it operated at the same frequencies as AMRAC III, the Rockwell System employed a filter with sin(x)/x characteristics that helped eliminate the problem with harmonics. The system proved to have a good signal-to-noise ratio (SNR), but crosstalk issues between substations became problematic.²

Now, the interest has shifted to bringing high speed internet to every corner of the globe. Yet, decades of new devices have put more noise onto the power line while they draw power and continue to make reliable high-speed, long distance communications elusive to engineers. In the future and even today, with advances in technology, these problems can be overcome.

IV. PRACTICALITY

A. Economics of BPL

When examining the overall feasibility of BPL, it is impossible to ignore the economic hurdles. If the cost far outweighs the utility of the system, it will not succeed. The Electronic Power and Research Institute (EPRI) looked at the economics of several BPL systems that currently exist in the United States and drew some conclusions.³ For infrastructure external to the customer's residence, implementation costs range from \$50 to \$150 per home. However, the in-home costs of power line modems and other appropriate hardware range from \$30 to \$200 per home. To remain competitive with cable and digital subscriber line (DSL) services, the implementation costs must be below \$20 per home for infrastructure and \$100 per home for connectivity equipment.

To bring the costs down, power companies could subsidize the service. Utility applications such as automatic meter reading and direct load control technology could be used to decrease cost to the power company. These savings could then be passed on to the consumer. If the cost becomes reasonable and the technology is comparable to other broadband options, BPL would have the advantage of an existing infrastructure and could succeed.

B. Regulations

As recently as October 2004, in an effort to promote broadband services, the Federal Communications Commission (FCC) revised its policies regarding BPL. In its Report and Order, the FCC defined BPL as any communications sent over a power line channel with a frequency between 1.705 and 80 MHz. Exclusion bands were set up to protect aeronautical receivers, and exclusion zones were determined to protect sensitive operations such as radio astronomy stations. In addition, the report mandates that BPL systems should be able to rapidly shift operating frequency bands and have a fast shut down procedure should any interference be encountered. Because BPL operates on an unlicensed, non-interference basis, specific standards for measuring interference levels were also defined.⁴

While the report was designed to further the development of BPL and to stimulate competition, there has been a significant backlash. There is a growing concern that BPL negatively impacts amateur radio operation. There is evidence that because power lines are unshielded for RF interference, they act as giant antennas that hinder radio signals. In response, the Amateur Radio Relay League (ARRL) has put out a call to "Stop the assault on ham radio!"⁵ As recently as March 2005, a BPL pilot project run by Amperion in Irving, Texas was shut down by the FCC due to pressure from the ARRL. Other Amperion systems have been shut down early as well, though they were deemed as successes. In part because of the ARRL's concern, many power companies have opted against implementing BPL at this point.

Though the concern of radio interference is valid, it is fair to say that the benefits of BPL far outweigh that of amateur radio. Broadband has the ability to allow for communication on magnitudes never possible with amateur radio. Nevertheless, the FCC insists that they are protecting amateur radio while encouraging BPL development.

V. THE POWER LINE CHANNEL: PROBLEMS AND SOLUTIONS

A. A Problematic Medium

The most basic characteristics of the power line itself are actually the biggest problems facing PLC and BPL; the network was not designed for frequencies other than 60 Hz. Because of this, the network is very inconsistent, and its parameters change over time, location, and load levels. This makes it very difficult to correctly model the attenuation, impedance, and noise on the line.

B. Frequencies of Interest

Over the years, experimentation with many different frequencies has been conducted with PLC. As previously mentioned, some of the earliest systems used frequencies below 10 kHz. An advantage here is that frequencies between 3 and 9 kHz are unallocated by the FCC and therefore less regulated. At these low frequencies, some signals can make it through a distribution transformer. This eliminates the need for transformer couplers and lowers the overhead cost. Unfortunately, because of the long period, data transmission is slow, and the harmonics of the 60 Hz signal are very destructive at this level. However, if these low frequencies were used in conjunction with new signal processing technology, it may

be possible and cost effective for power companies to use this region for such applications as dynamic load management.⁶

In the next frequency range, from about 10 kHz to 2 MHz, the power line takes on a whole new set of issues. At these ranges, other licensed frequencies need to be considered. In this range there are many other communications in use, most notably the AM radio band. This makes any PLC system susceptible to noise and conversely, makes these other communications susceptible to noise from the PLC system. Consequently, this frequency band's major disadvantage is noise. Although utility PLC systems would work effectively here, it is not ideal for BPL.

The best frequency band for BPL is between 2 and 80 MHz. Noise is less of a problem than in the previously mentioned band, but attenuation now becomes a factor. In addition, the BPL signals create noise on amateur radio bands contained within this range.

C. Impedance

The impedance of the electric distribution system is quite difficult to characterize. It is defined for a given frequency and can range anywhere from a few ohms to a few kilo-ohms. Depending on a given load and the network topology, the impedance can generally be characterized somewhere between 90 and 100 ohms.⁷ However, the low-voltage residential circuit is more difficult to characterize.

Vines et al.⁸ experimented with line impedance in the mid-1980s and were able to draw conclusions at low frequencies that Pavlidou et al.⁷ were able to adapt to higher BPL-capable frequencies. In measurements from 5 to 30 MHz, the following was determined:

• The magnitude of the impedance increases with frequency in the range of 5 to 20 MHz.

• The mean value of the impedance increases from about 5 Ω at 20 MHz to about 120 Ω at 30 MHz.

• There is a strong fluctuation between the maximum and minimum value of the impedance.

• Resonances can occur on the residential network above 40 kHz. This makes the impedances of higher frequencies more unpredictable.

• Of all load types, resistive heating coils cause the most major changes in overall impedance.

Although a statistical model is difficult to develop, the impedance of the medium voltage network can be calculated based on typical loads, lines, and transformers. Beyond the transformer, on the low voltage network, the impedance can be best characterized by a 5 Ω H line impedance stabilization network (LISN) as pictured in Figure 1.9. Both Vines et al.⁸ and Nicholson et al.⁹ agree that this is the best working model to describe line impedance.

D. Signal Attenuation

Over distance, as power lines weave their way under and above ground, any PLC signal coupled to the line will attenuate. Over a typical U.S. distribution line, BPL will only travel less than a mile before amplification is needed.¹⁰ In the mid-1980s several engineers experimented with the power lines that existed in their labs and surrounding neighborhoods.^{1,11,12} While most research done on attenuation over power lines was done twenty years ago, most of the data is still relevant



Fig. 1. Line impedance stabilization network schematic (LISN).

because the grid does not change substantially over time. After testing the frequency range of 20 to 240 kHz, Chan et al.¹¹ came to several conclusions. First, signal attenuation on intra-building power lines usually exceeded 20 dB, except for over short distances. Also, when the transmitter and receiver are used on different phases, the signal does actually couple between the phases, but with much higher attenuation. At higher frequencies, up to 240 kHz, attenuation was usually more severe. In addition, narrow-band signal fades can occur at specific signaling frequencies. Probably most noteworthy was how load affected attenuation; depending on the time of day, the attenuation could vary drastically, as shown in Figure 2.¹²

If we extrapolate these results, we can see that at the higher frequencies necessary for BPL, attenuation is certainly a problem. Amplifiers or repeaters could always be used to boost the signal, but that would require infrastructure improvements, reducing the cost-effectiveness of BPL. Nevertheless, placing repeaters every mile or so seems to be the best way to overcome attenuation.

General line losses are not the only way a PLC signal is attenuated. Distribution transformers are a major culprit. By their very design, only low frequency signals will pass through them unobstructed. If a high frequency does manage to get through, it is nearly impossible to reconstruct.

Therefore, to overcome transformer attenuation, one of three methods must be employed. The first two methods involve bypassing the transformer completely. The signal can be extracted from the medium voltage line and sent via RF signals directly to neighboring houses. The other option is to extract the signal from the medium voltage line, convert it, and inject it onto the low voltage line. The third method is to push the signal through the transformer using direct sequence spread spectrum modulation, which is similar to orthogonal frequency division multiplexing (OFDM) but is more reliable and requires greater power. However, the signal would still be attenuated and repeats would be required.³

E. Noise

Noise is easily the most destructive force on communications sent over the power line. On high voltage networks everything from lightning to switching circuit breakers adds noise to the lines. On the medium voltage network, power-correcting capacitors are the major culprit. This means that BPL would need to function best on the low voltage network where the most noise exists due to consumer appliances. In general, the



Fig. 2. Attenuation versus frequency, industrial building, daytime (top), nighttime (below). IS represents a short distance on an in-phase channel. I represents a longer but unknown distance on an in-phase channel. A1 and A2 are across-phase channels. It can be seen that the attenuation of the power line channel varies greatly based on time of day. This is due to daily load changes.

noise is lowest at higher frequencies, as indicated by Figure 3. This is beneficial for BPL, which needs to operate at high frequencies to be useful. The noise on the low voltage network can be classified into four categories: synchronous impulse noise, "smooth spectrum" noise, single event impulse noise, and asynchronous background noise.^{1,12,13}

1) Synchronous impulse noise

Synchronous impulse noise occurs at multiples of 60 Hz, has a duration of a few microseconds, and a power spectral density (PSD) that with line spectra at 60 Hz multiples and decreases with increasing frequency.¹³ A main cause for synchronous noise is silicon-controlled rectifiers (SCRs). A SCR switches when the voltage reaches a certain level, and because the voltage is cyclical, the SCR switches on multiples of 60 Hz.

Another source of synchronous impulse noise is solid state light dimmers operating at the main frequency. While these devices should comply with frequency compatibility issues, standards for frequencies below 500 kHz are unspecified.¹²The voltage and current characteristics for a common light dimmer can be seen in Figure 4. It can be seen from these plots that the interference is indeed synchronous with the power line.

Because the noise is synchronous, it is much easier to predict and therefore much easier to filter out. As Tengdin notes in regard to the AMRAC III system, "by the use of a receiver filter with $\sin(x)/x$ characteristics phase locked to the power system frequency, which has nulls at the harmonic frequencies (Figure 5), it [is] possible to operate in these relatively quiet valleys between the adjacent harmonic peaks."² This method



Fig. 3. Simulated power line channel model.13 While only frequencies above 200 kHz are noted, it is still possible to transmit in lower bands, but clearly noise become more problematic at such bands.

essentially removes the peaks due to the 60 Hz harmonics but can also be effective at removing noise from other devices on the network.

2) Smooth spectrum noise

Smooth spectrum noise is simply the summation of all low-power noise sources. Its PSD, while higher than the PSD of synchronous noise, is relatively even with no line spectra at specific frequencies. Smooth spectrum noise is primarily caused by line loads running asynchronously to the power line frequency. These loads are generally universal motors where the speed of the motor is controlled by the current rather than the voltage. Some of these appliances include electric drills, vacuum cleaners, mixers, blenders, sewing machines, and electric saws.

Over small bandwidths, noise of this nature can be modeled by band limited white noise.¹² In this case, a simple digital signal processor (DSP) would be sufficient to model this noise and filter it out of the received signal. In addition, "forward error correcting codes combined with interleaving (to provide time diversity) [could] be implemented" to deal with the effects of this noise.¹ This method is also effective in coping with synchronous impulse noise.

3) Single event impulse noise

Single event impulse noise presents the greatest challenge to PLC. The duration can vary anywhere from a few microseconds to a few milliseconds, and the inter-arrival time is completely random. The PSD is quite high and can be up to 50 dB above the background noise spectrum.¹³ This noise originates from transient sources such as switching thermostats, power correcting capacitor banks, or even lightning.

Because this noise is so unpredictable, it is difficult to filter. It can be modeled as impulses, and similar impulse noise on other communications can be overcome by the error correcting code mentioned previously.

4) Asynchronous background noise

Also called narrowband background noise, asynchronous background noise refers to a PSD with specific line spectra that is unrelated to the main frequency. The interference varies based on the time of day but originates from three primary



Fig. 4. Voltage across (left) and current though (right) power circuit with dimmer set for maximum brightness.12 It can be seen where the SCR in the circuit opens early and closes late, causing interference.

sources:

• Televisions: televisions emit a synchronization pulse every $63.5 \ \mu$ s, which consequently creates a large amount of noise at $15,734 \ Hz$ and its harmonics.

• Computer monitors: like a television, cathode ray tube (CRT) computer monitors also emit a synchronization pulse, but the period is not standardized and varies by manufacturer.

• AM sinusoidal signals: some AM radio stations have historically broadcast their signals over power lines as a secondary transmission method know as carrier current. These stations, as well as short wave and amateur radio, can cause notable noise on the line.

To avoid asynchronous background noise, the best option seems to be to avoid problematic frequencies all together. As such, the base television frequency of 15,734 Hz and its harmonics should simply not be used. However, this does not account for unknown monitors and AM signals. Ferreira et al. suggests using some sort of frequency diversity such as frequency hopping along with error correction to deal with the line frequencies of computer monitors.¹

To counter noise and reduce RF interference, an appropriate adaptive modulation scheme implementing DSPs must be used. Ten years ago, DSPs did not have the computing power to overcome the power line noise, but that is no longer the case. Current DSPs are faster and more powerful than their predecessors. They can now clean up the signal mathematically and adapt to real-time conditions on the power line.

Additionally, in the past two years, new modulation schemes have been developed that have proven to be quite successful in PLC.¹⁴⁻¹⁶ Specifically, experiments with frequency shift keying (FSK) and OFDM have shown promise.



Fig. 5 Frequency response of sin(x)/x filter phase locked to the power system .2 Using this method, the 60 Hz frequency and all harmonics can be exclusively filtered on the receiver-side.

Frequency shift keying has existed since the mid-1900s and is a simple modulation scheme conceptually. In the simplest method, binary FSK, two different frequencies are utilized to send a single digital signal. Basically, a logic "1" is sent as a sine wave over one frequency while a logic "0" is sent as a sine wave over a different frequency. This makes the modulated signal less susceptible to noise

However, there are drawbacks to FSK. The maximum successful transmission speed is relatively slow compared with that required for broadband. As such, FSK would be more useful for metering and load control applications where speed is not a factor. In addition, frequency selective fading is a problem. In other words, deep notches appear in the frequency response and can vary over time, cable, and location. If the signal is modulated to one of the faded frequencies, performance is severely degraded. Also, because line attenuation increases with frequency, "many bands are not flat enough to accommodate high-rate communications with narrowband modulation."¹⁴

OFDM is a spread spectrum modulation scheme. The transmission signal is split into several narrow-band channels and modulated to different frequencies and phases. Unlike standard frequency division multiplexing (FDM), priority is given in minimizing crosstalk between the channels rather than trying to perfect each channel.

There are several benefits to OFDM: it is resilient to RF interference, has a lower multi-path distortion, and a high spectral efficiency.¹⁷ It has also proven highly effective against high noise levels and narrow-band interference, which are BPL's biggest foes. However, within the range used for BPL OFDM (1 to 100 MHz), there is no FCC license, thereby limiting the power level and causing greater attenuation. Nevertheless, OFDM is certainly an effective and viable method for BPL.

VI. BPL TECHNOLOGIES IN PRACTICE

One indication that BPL is imminent lies in the fact that several companies have jumped on the PLC bandwagon. Power line modems, while primarily designed for intra-home networking, are commercially available from such major electronics manufacturers as Linksys, Belkin, Netgear, and others. In addition, there are now a handful of vendors that are promoting BPL in about twenty-five pilot cities.¹⁷

The Ambient Corporation specializes in BPL system design and integration. They send signals onto the medium voltage lines and a patented coupler to transfer the signal to the low voltage network. Repeaters are used where necessary.

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Consolidated Edison and IdaComm are currently testing equipment made by Ambient.

Massachusetts-based Amperion, Inc. specializes in BPL over the medium voltage network. At the distribution transformer, the signal is then converted to a Wi-Fi signal that can be accessed by customers. Additionally, there is an optional fiber connection that can be run from the transformer. They have seen successful raw data speeds of up to 45 Mbps. Amperion technology is being deployed at AEP, PPL Telecom, Progress Energy, IdaComm, Dacor, and several others.

Current Technologies in Maryland offers everything from the hardware, software, installation, operation, and full ISP services of a BPL deployment. As such, most of Current Technology's system is proprietary. Current pilot projects are being conducted at Pepco, Cinergy, and Hawaiian Electric. Cinergy's implementation has speeds ranging from 1 Mbps (\$29.95 / month) to 3 Mbps (\$39.95 /month), and its clientele has surpassed 55,000 homes.³

While the previous technologies are primarily being piloted in cities, GridStream Systems in Tennessee focuses on system design in rural areas. Their system imposes a signal between the phase line and neutral line of the distribution circuit, and employs a patented modulation scheme. It is currently being tested by Cullman Electric, Fayetteville Public Utilities, Chelan County, WA, and Winchester, TN.

Main.net Powerline Communications, Inc. has conducted experiments on many of its resources in Europe. Main.net specializes on BPL system design and integration. Its parent company is based in Israel and there are about fifty deployments outside the United States. Within the United States, its primary pilot city has been Manassas, VA, serving over 15,000 homes with 800 kbps service at \$26.95 per month.³ Other customers include AmerenUE, Rochester Public Utilities, and Pacific Gas and Electric.

California-based PowerWan, Inc. has developed a system that eliminates the need for a coupling between the medium and low voltage networks. Their signal processing technique detects a signal after passing through a transformer. Their test level is fairly low with "'unnamed utilities' serving more than a dozen homes."³

VII. CONCLUSION

It is clear that even though BPL is a difficult prospect, the advantages far outweigh the problems. The history of PLC has been dotted with mild successes, but its future is much more promising. New technology is allowing the problems of impedance matching, attenuation, and noise to be overcome and avoided.

However, because of the necessary capital improvements, it is still difficult to get utilities to take on the risk of implementing a trial program. Nevertheless, in the few places where it has been tried, according to the individual companies, it has been quite successful. There is no doubt that as technology improves, the broadband market will become more competitive and prices will fall, allowing BPL to step in as a major contender.

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Reid L. Sprite, an alumnus of the University of Rochester, completed an independent study major in the Electrical Engineering discipline. After graduation, Sprite now works as an Operations Engineer at National Grid in Malden, MA.

jur: What is electrical engineering research all about?

While my research in high-speed communications over the power line is very broad-based, in the new economy, easy access to the Internet is essential. Today, power lines connect nearly every corner of the globe and have created the most widespread electrical network. However, the power line presents many challenges to communication signals, which is the basis for my research.

jur: Who or what motivated you to do this research project?

I grew to have quite a strong interest in power engineering and wanted to create an independent study course that would suit my needs. After talking with my faculty advisor, Professor Roman Sobolewski, he agreed to also serve as my advisor for an independent study course. In an effort to find a specific power engineering topic that both he could advise on and I was interested in, we decided on examining the future of broadband over power line.

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

As I mentioned, I already had an interest in power engineering and had planned to enter that field. Fortunately, I found just the job I was looking for as an Operations Engineer at National Grid.

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

Although my research on BPL involved very little experimentation, my related senior design project gave me first hand experience of some of the problems facing BPL. The power line such a mixed bag of resistance, capacitance, and inductance that is very hard to model what will happen to signals imposed on the lines. The best options to overcome this was to closely examine many experiments done by other experts that included various signal modulation techniques as well as specialized filters.

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

My goal of the research was to gain a new understanding for the complexities of the nation's power grid. I learned much more about how the grid is constructed and maintained, which will be invaluable knowledge in my new career.

jur: Any advice you could give to fellow undergrads who would like to undertake similar research (or any research in general)?

The best advice is to not always overthink. Do not reinvent the wheel. You may have a small problem within your larger project that has you stumped, but it often seems to be the case that someone has already encountered and possibly solved it. By hitting the books and finding that useful research, it will help keep your project on-track and on-time.



O Captain My Captain:

The Relationship Between Officers and Enlisted Men in World War One

Susan Thyne, 2006

Advised by Stewart Weaver, Ph.D.

Department of History

hile the study of war is generally on a grand scale, soldiers more often remember the war for the experiences with the men next to whom they fought, or the men they followed into battle. As historian Eric Leed has said, "The analysis of war as a social experience and a human phenomenon has been skewed by the historian's interest in the 'significant.'"1a Dates, defeats, and victories are significant, but so is the relationship between officers and their men. Indeed, it appears that their experiences together in the First World War subverted this social distinction. By examining the British army, we find an interesting juxtaposition of upper and middle class junior officers and common enlisted men ignoring the social barriers present in peace time, adapting to trench life, and catapulting themselves over the parapet to win the war for their comrades and country. Their personal letters and diaries reveal their true thoughts about each other: that men were willing to follow their officers to the ends of the earth (or the ends of their lives) and officers were moved and motivated by their men's willingness to fight. Without the relationship that developed, the British army would not have been as successful as it was in fighting the war.

The background of the officers and men is part of what made their relationship unique. Officers belonged to the upper and middle classes, most likely gentlemen; enlisted men were regulars in the British Expeditionary Force, Britain's army for rapid deployment, or volunteers and conscripts from all walks of life. The two social classes were distinctly different and each possessed a set of characteristics unique unto itself.

The officer class recruited for the war was officially upper class. Prior to the war, all officers in the army were recruited from British society's elite, and once war broke out, these men continued to serve in higher positions among the senior officer staff. The officers closest to the men, junior officers, are largely remembered as the "Public School Officers" since most received their training in the Officer Training Corps (O.T.C.) at boarding schools. Historian John Keegan sums up the qualities of these men: "Officers had to be gentlemen...though education at one of the public or better grammar schools which ran an O.T.C. was in practice often found sufficient..."^{2a} These broader qualifications for officers opened their ranks to men from the middle class. Many arguments have been made as to why these officers, most coming from the academic world, could assimilate into the role of commander. Most discussions, however, generally conclude that the competitive spirit and structure of the public schools was so similar to the army's that there was a natural transition from school to army: "For the British regiment, with its complex and highly individual accretion of traditions, local affinities, annual rituals, intercompany rivalries, fierce autonomy and distinctive name... was an extension, indeed a creation of the Victorian public school system."^{2b} Although these public school officers could assimilate into army life and structure, they brought a distinctly civilian approach to discipline and built relationships with their men just as they would with schoolmates.

While the officers of the upper class enjoyed the competitive games of the public school system, the newly enlisted men came from a different world. Historian Peter Liddle draws attention to the mistake of categorizing all the new men together: "It may be dubious to put forward an individual pre-1914 soldier as representative of his fellows...because so many factors might distinguish that person from those whom he might otherwise properly represent. Motivation, social background, and military rank are merely some of the variables..."3a Territorials, those men who served in the British Expeditionary Force, mobilized at the beginning of the war served in the reserves, training after work, on weekends, and in the summer. New volunteers left jobs of all sorts (or no job at all; many men were unemployed in the summer of 1914 and found the army an appealing alternative to poverty), as well as wives, families, homes, and other such domestic responsibilities. These volunteers poured into recruiting centers by the thousands, called there by the new Secretary of War, Kitchener, to serve Great Britain. Many joined in groups known as the Pal's Battalions (the men were promised that those who signed up together would stay together), while many more were complete strangers to one another.4

Whether the men knew each other or not, the British armies became one great melting pot of class, society, and geography.

It was almost always a meeting of strangers. It was sometimes a meeting of near foreigners...When nicely raised young men from West Country vicarages or South Coast watering-places came face-to-face with forty Durham miners, Yorkshire furnacemen, Clydeside riveters, the two sides found that they could scarcely understand each other's speech.^{2c} Despite unfamiliar dialects, a common cause united the army, and while the collision of these two worlds was a large factor in determining the relationship between men and officers, it must be remembered that the worlds came together in the close confines of the trench. In this maze of earth, men lived, worked, ate, rested, fought, and died. The trench, therefore, created a backdrop for the melting pot, an entirely new world for each and every man, and a new order by which to live.

The trench, for which the First World War is chiefly remembered, was home to the men for four years. Any account of the war includes a description of the trench system, the maze of ditches that both protected and trapped soldiers and brought the war to a stalemate. However, when Liddle describes the trenches, he cautions against the precise definition: "It is unwise to generalize about conditions in front-line trenches because so much would depend upon the geography, the season, the weather, the degree of military activity."3b However, it is true that the trenches were typically six feet deep and the same in width. Sandbags were stacked brick-like as a front wall above the opening, and a firestep allowed for observation or the launching of an attack. Trenches did not continue in a straight line for very long; a zigzag pattern developed to minimize the effect of a direct hit in a trench. Dugouts in the side of the trench offered protection and a place to rest, and second and third lines were connected to the front by communication trenches that ran perpendicularly.

Ideas about the impact of trench warfare are plentiful, but A. E. Ashworth's research has drawn much attention. In examining the effects of the trench, he notes several points, the first being that "the physical structure of the trench ensured that the activities of the ordinary soldier, for some part at least, were not visible to their officers... [This] ensured that the frontline soldier was protected against over vigorous surveillance by his officers."^{5a} This point is often overlooked by the casual observer and must be remembered when examining first-hand accounts of interaction between enlisted men and officers. A high degree of trust must develop in such situations when a superior cannot always keep watch. At the same time, the subordinate must be instilled with a sense of duty in order for the integrity of the system to remain intact.

In addition to trench structure, Ashworth's other point regards morale. The war was supposed to be the Great Adventure, but entrenchment ironically immobilized the armies shortly after mobilization. Officers were to keep their men motivated by instilling a fighting spirit in them and keeping them in the offensive mindset. While there was a considerable schism between senior staff officers and the frontline, Ashworth points out that "one might expect the officer, other ranks dichotomy to have...significance in the trenches. However, this was not absolutely so...Combatant officers together with other ranks shared a situation of danger in the front line."5b Perhaps the sense of duty the soldiers felt came from the fact that they were not facing the peril of the trench alone and instead their superiors, the junior officers, knew exactly what the men faced. This common environment alone would help to raise morale. Between the development of the trenches and the origins of the men serving in the army, we begin to see the foundations of the relationship between men and officers.

Fundamental to the relationship was the way in which

officers viewed their men and vice versa. Before a man had to prove himself or his worth, he simply had to be in the trenches. There, amidst the dirt and death, relationships were simple. Historian John Baynes, who himself served during the war, studied a British battalion and points to the basic nature of the war:

The main cause of this wonderful relationship between good officers and their men in the trenches was that all the trappings of life were removed, and the real worth of an individual was revealed. All the aspects of normal life which divide men from each other became of secondary importance...where a man came from and where he might go after the war was of little account; wealth, background, and education only mattered in as far as they had fitted a man to play his part in the structure of the battalion; whether he was a saint or a sinner nobody cared, but only that a man could do his job.⁶

While each man was aware of social status in peacetime, the trench eliminated social barriers and revealed each man's true worth.

The writings of men and officers in the war help further illuminate their relationship. Both officers and men offered high praise to those they deemed worthy, which in most cases, was all with whom they came in contact. Second Lt. Robert Wallace McConnell wrote to his father that "the men are all topping fellows,"7a while another second lieutenant, Lionel Sotheby, wrote in his diary that "the ordinary Tommie is a most extraordinary fellow...It can be said, however, with absolute truth that he is the most wonderful, the enduring, and the most devil may care human being in existence."8a The sentiment is shared by Captain Ivar Campbell who wrote, "There is one thing cheering. The men of the battalion...are cheery. Sometimes, back in billets, I hate the men...but in a difficult time they show up splendidly."7b Similar entries in journals and letters by officers can be found in reference to their men. Generally, the men were perceived as good, willing fighters who put up with the worst to join the war effort. An excellent observation of officers comes from John Gibbons who served as a private during the war. In his book he writes, "As it is, from the War-time private's point of view the officer's quality chiefly comes out in the actual fighting, and I should have said that in the Line the officers on the whole were slightly better than ourselves."9a Later, Gibbons writes, in a passage that seems to mirror Baynes' idea that a man's worth was judged by the kind of job he did in the field, that

...we were more or less friends with our sergeants, some men more so and some men less; in general if the private soldier did his very easy job fairly properly and did not get the name for any too obvious dodging of duties and letting down his neighbors, then a great deal would be forgiven him, and his relations with his non-commissioned officers would be quite fairly decent.⁹⁶

While Gibbons' description of his officers is not as colorful as the officers' opinions of their men, it certainly displays the same sentiment: unexpected admiration for each other.

Although the trenches subverted the peacetime social hierarchy, the backgrounds of both men and officers affected their behavior. In commenting on his captain, Gibbons wrote, "I believe that normally he was a solicitor with a suburban practice, where opportunities for self-expression would presumably be rare."⁹ Here, the war allowed the upper and middle classes to break free from the restraints placed on them by social norms and expectations; in the trenches, an officer

could live up to his potential amongst his men as long as he maintained discipline. Officers also noted that social class influenced the attitudes of the men. Lt. Hugh Butterworth wrote home to his colleagues that the men "will go like lambs as long as they've got an officer with them. The curious thing is that in civilian life they've probably cursed us as plutocrats, out here they fairly look to us."7c For many officers, it was not just the opportunities to lead their men that brought them to the realization that their men saw the world differently. In censoring their men's letters, the officers had an eye-opening experience that revealed the lives from which the men came. On censoring, John Manwaring wrote, "What an insight into human nature a day in the Censor's office gives one. Here one touches as it were upon the fringe of human emotion, one reads the thoughts of those with whom one would never come in contact."10a By reading his men's letters, the officer further connects with his men. Manwaring understood that neither he nor his men would be the same after the war and being exposed to other parts of society. He further wrote,

Oh, the many sides of life that lie in these soldiers' letters! The pathos of the men whose dear ones are lying ill at home; messages to children they have never seen and may never see; words of comfort and hope to mothers, fathers, sisters, brothers, wives and sweethearts... Truly one dips below the surface of life, and we who have seen all this can never be the same careless pleasure-seekers again.^{10b}

Regardless of whether social backgrounds were apparent in how men acted, the trench brought a level of equality unparalleled in British society.

The trench may have deemphasized class structure, but at the same time, upper class officers helped to foster a sense of equality. Leed theorizes that:

...in this war those sons of the bourgeoisie who were initially defined by their idealistic expectations became familiar with activities and human relationships that were wholly unfamiliar to them...but perhaps the most significant effect of the social experience of war was the diminution of both the guilt and the romanticism with which morally uncomfortable sons of the bourgeoisie had formerly regarded their social inferiors.^{1b}

In war, class conflict was no longer an issue. What mattered was the will to succeed.

Surviving trench warfare was not the only goal, nor was it necessarily the main goal; winning the war had supreme importance. Officers received training in keeping up men's spirits and in boosting morale, but the ultimate goal was getting men over the parapet in an attack. Here lay the heart of the relationship between men and officers: enlisted soldiers had to be willing to follow their officer onto the battlefield. With their officers as their ultimate inspiration, the men of the British army were willing to leave their trenches and bring the army to success. Keegan characterizes the leadership as more important than the fellowship between ordinary soldiers and argues that

...self-confidence and credulity were certainly present, and powerfully effective at persuading the Pals to jump the parapet. But to emphasize the populist character of the Kitchener armies is to minimize the importance which leadership played in taking it into battle. And arguments can be found to suggest that leadership – conscious, principled, exemplary – was of higher quality and greater military significance in the First World War, at least in the British army, than before or since.^{2d}

Gibbons cites this model of leadership as part of his reason

for not wanting to be more than a private: "[the officers] had an outward and visible standard of courage that I think was higher than our own. As a coward, I could just manage to do as I was told and to keep myself from running; I do not think that I could have managed the responsibility of other men's lives or the standing up by myself.^{"9d} This admiration for the job the officers accomplished carried a soldier far. Keegan cites an officer's opinion on the criterion for a successful officer: "Were they or were they not braver? That was your criterion...For the act of being brave compelled the utilization of the whole reserve of moral force that lay in a man...every battalion had its own little core of officers around which the battalion clung. Wounds or sickness might get them but sure enough they'd return..."^{2e}

The admiration men had for their officers' courage was returned by the officers in their admiration for their men's persistence. One officer wrote that men coming off of the battlefield had a certain look about them, and it was understood from where they had come. Beyond their physical appearance, though, "a triumphant smile on their haggard faces tells of a duty well and truly done. They have cut their notch still a bit higher, and have earned their rest, as well as their place on the scroll of fame."7d Officers were quick to give credit where credit was due. Sotheby similarly praises his men after an attack: "It was truly wonderful...Not a man hung back, all charged as far as possible. A finer set of men than these, and mostly Reservists, could not be found anywhere."8b In these short descriptions of battles, officers rarely wrote of their own courage or deeds. Instead, their letters and journals relate their admiration for their men independent from their own leadership.

In the privacy of correspondence, officers doled out their highest praise and deeply personal feelings for their men. One captain wrote of possibly changing companies, but both his superior officer and his servant did not want to work with anyone else; the captain was pleased to learn that "evidently my love of men is not wasted here. I think I know the ways and peculiarities of every man of mine; it surprises them, and they like it and work well for it."7e Another captain gives similarly high praise to his men: "What impresses and moves me above all is the amazing faith, patience and courage of the men. To me it is not a sort of looking-down-on but rather a lookingup-to appreciation of them. I pray and pray and am afraid! - they go quietly and heroically on. God bless them and make me less inferior to them ... "7f One would be hard pressed to find an instance of an officer making such a statement in any context but private writings. Furthermore, Sotheby writes of being removed from his men by a promotion to command several companies for almost two weeks but is uninterested in continuing: "...I have no wish to be anything than a second Lieut. out here - one is part of the men themselves then, and that is what I like."8c Unable to display their admiration and affection for their men in the trench, the officers' writings become our best source for understanding their true feelings for their men.

But was it only admiration and the primitive nature of the trench that helped the relationship develop? Certainly not; the officer was paternalistic, but even more so, he was a Christ-like figure. In support of this, many historians cite a prominent passage by Donald Hankey from an essay for the Spectator in which he refers to the ideal leader in the context of being the soldier's Christ.^{2e} Officers walked the same ground as the men, lived with the same danger, and yet took the time to make sure even the simplest comforts were provided. Men did not simply admire their officers; they worshipped and exalted them, and for this, the men were more willing to follow their officers onto the battlefield.

Other hopes of a utopian world extending beyond the trenches arose. Here, where every man was equal, the men and officers could be comfortable in their relationships since they were necessary for survival, not merely for military structure. Leed cites the socialist F.H. Keeling, who believed that "soldiering was a ritual that he celebrated as a kind of civil religion to be prized precisely because it was the antithesis of the privacy, individuality, and family-centeredness of civilian life."^{1c} Keeling goes so far as to call the trench-phenomenon "communistic." The sentiment was not confined to socialists alone, but rather it was supported by concurrent social change in Britain.

A rising public literacy and an expanded role for public opinion in government also played their roles. For some an expectation arose that when the working-class Tommys and the upper-class Nigels fought together for England there would be social egalitarianism as well. Despite differences in class, ethnicity, and background, the comradeship of a volunteer army engaged in the great cause of national honor would create an almost utopian social world, or so the hopes went.¹¹

Regardless of postwar social movements, we can conclude that the equality developed in the trench overpowered any class segregation, and men saw past social distinctions in an urge to survive, cope, and win.

Class distinctions shaped attitudes towards each other, but the melting pot that was the British army and the danger of the trenches strengthened the bond between men and officers as a sense of equality took hold. The experiences of World War One subverted any distinction between officers and men, and we are left today with a legacy of admiration and motivation that propelled men to leave the relative safety of the trench and follow their officers to no man's land.

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About the Author

Susan Thyne, a senior majoring in History, did her research for her major seminar under the guidance of Dr. Stewart Weaver. Susan plans to take a year off to work after graduation before attending graduate studies in Library Science and History

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

This researched examined social relationships in the British army during World War I. War is more than just dates or battles and this research strives to prove that war is significant to the individual for very different reasons: in this case, it was the encounters between social classes that both enlisted men and officers remembered and repeatedly mentioned in their personal writings. While I examined only the war period, this research could be followed with research about British society in the interwar period and how class culture began to change.

jur: What motivated you to do this research project?

This project was for the History major seminar and the idea for the topic was sparked after reading Robert Graves's war memoir, "Good-bye to All That."

jur: What was the biggest obstacle of this project and how did you overcome it?

The hardest part of the project was finding secondary resources that related to the specific topic. I wasn't sure how to go about using different databases to find articles in different journals. I met with Alan Unsworth, the History librarian, and he helped me navigate different databases and gave me many ideas about how to search for what I wanted.

jur: Any advice you could give to fellow undergrads who would like to undertake similar research (or any research in general)?

This research has taught me a few key points. First, the narrower the topic, the better your research will be. It's easier to start with a small idea and be able to build on it as you find information in your research; it's much harder to start with a vague generalization and expect to find specific evidence to support your argument. Second, after working with Alan Unsworth, I've realized how much easier research can be if you work with a librarian. By not tapping a librarian's knowledge, you're making your own work harder; the librarians know how to find the resources you need much better than you do.

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A Comparison of Litter Densities in Four Community Types of the Long Island Central Pine Barrens

Dana Tievsky, 2007

Advised by Timothy Green, Ph.D.

Brookhaven National Lab

The Long Island Pine Barrens Society was founded in 1977 in order to bring attention to the depleting natural resources of the Pine Barrens. The Long Island Pine Barrens is a unique mosaic of forest types that contains the purest drinking water and greatest diversity of plant and animal species anywhere in New York State.

In the 1970's, with the realization that residential and commercial development was disturbing and potentially destroying the area, conservation efforts began. Initial preservation attempts to provide core or "greenbelt" areas, shown in figure 1, during the late 1970's and early 1980's did not alleviate threats to the Pine Barrens ecosystem. After many failed attempts, protective legislation was enacted in 1993 with the establishment of the Comprehensive Management Plan in 1995.^{1,2}

In 2003, the Foundation for Ecological Research in the Northeast (FERN) was founded to fund ecological and environmental research at Brookhaven National Laboratory.⁴ The primary project of FERN is the Central Pine Barrens Monitoring Program. The goal of this project is to track the current and future health of the Pine Barrens so that future research needs and priorities can be identified.³ Since little is known about the exact ecological status of the Pine Barrens, it is anticipated that this data will be crucial to a wide spectrum of residents and organizations including researchers, developers, environmentalists, and state and local government. Since the Pine Barrens is a natural feature unique to Long Island, it is critical to keep this resource healthy and thriving.

It is anticipated that the results of this research will provide data relevant to the determination of appropriate timing for prescribed forest fires. Properly timed wildfires benefit the Pine Barrens. Reduction of litter (which is composed of leaves, twigs, pine needles, and other dead vegetation) and canopy cover in the forest provides for direct sunlight on the soil and triggers new tree growth. Furthermore, pitch pine germination is augmented after fires. Melting of the resin coating enables the cone to burst open and scatter seeds directly on bare soil.^{5,6} Knowing the right time to prescribe forest fires would not only better the health of the Pine Barrens, it would also increase their longevity.

Baseline data for this longitudinal study were collected during the summer of 2005 at Brookhaven National Laboratory. Pitch Pine, Pine-Oak, Oak-Pine, and Coastal Oak forests were targeted at this time. Pitch Pine forests commonly have a canopy cover of nearly 100 percent pitch pine trees while Pine-Oak and Oak-Pine forests have a canopy of mixed pitch pine and oak trees. All these community types include a shrub layer consisting of huckleberry, blueberry, and scrub oak. Coastal Oak forests typically contain a canopy of various tree oaks and little to no pitch pines in addition to "a nearly continuous shrub layer of huckleberry and blueberry."³

In order to describe one aspect of the succession of the Pine Barrens, litter depth was measured in each of the four community types.

Materials and Methods

Plots in the Central Pine Barrens throughout eastern Long Island were randomly selected using a Geographic Information System (GIS). Each plot was first located using a Global Positioning System (GPS) to insure that it was in the targeted community type and more than 50 meters from disturbed areas such as roads, wetlands, and other plots. Once the 16 x 25 meter plot was accepted, the boundaries were marked with measuring tapes. The 25-meter edge was placed parallel to any apparent data-influencing factor such as topography and human disturbance. A sighting compass and a laser rangefinder were used to assure precise measurements.

Next a random starting point was chosen along the 16meter tape to position the first of ten line transects, each 1.5 meters apart. Another random number determined the starting point for data collection on each transect. Shrub, tree, and herbaceous cover was recorded at twenty points, each one meter apart, along every transect. A narrow tent pole was used to determine each "hit" point and the phenology (fruiting, flowering, or neither) of each plant was recorded the first time it was "hit" on each transect. For example, one point might include litter, huckleberry, and scrub oak so three items would be recorded for that "hit" point. A densitometer was used at each point to determine an exact reading of the canopy cover. The canopy cover was recorded as "pine," "hardwood," "both," or "nothing." Litter and duff depths were measured to the nearest millimeter at points 3, 8, 13, and 18 along each transect. (The duff layer is a dark brown soil resulting from decomposed stems, roots, and charcoal between the litter and the mineral



Figure 1: A map of the Central Long Island Pine Barrens indicating the core preservation area.⁹

soil, which is usually gray or yellow.) Litter was measured by dropping a ruler into the ground until a firm surface was reached. A soil corer was used to measure the duff layer.

Belt transects were completed following the line transects. Tapes were placed at two, four, six, and eight meters along the sixteen-meter edge of the plot so that seedling and sapling data could be collected for four belt transects. Saplings that were greater than 2.0 meters tall and less than or equal to 2.5 centimeters (cm) in diameter at breast height (dbh) were tallied separately from those that were between 0.5 and 2.0 meters tall. This means the tree's diameter was measured at about 1.3 meters above ground. Tree seedlings less than 0.5 meters tall were recorded by species and placed into the following categories by number of seedlings: "1 to 5," "5 to 10," and "greater than 10." Since more than 10 seedlings per belt transect corresponds to exceptional regeneration, 11 was the cap counted per species. Multiple stem plants were only counted once and scrub oak seedlings were not counted.

Next, data on trees, snags, and downed logs were collected. The diameter at breast height was measured for all trees greater than 10 cm dbh and data were recorded by species. Trees greater than 2.5 cm and less than 10 cm dbh were tallied by species. Trees with multiple stems were counted as one tree, but the dbh of both trunks was measured and recorded. Downed logs greater than one meter in length and 10 cm dbh were measured in length and dbh at the middle and both ends.

We also estimated the percent cover and average height of each stratum including trees, shrubs, vegetation, and epiphytes. The slope and aspect of the plot were also measured using a clinometer and a compass. The edges and center of the plot as well as a witness tree were marked and GPS coordinates recorded so that the plot could be located in the future. Digital photographs were also taken from the center to each corner of the plot.

A total of 40 plots were measured, but 10 were excluded from the study due to the intermediate nature of the community type. The breakdown of the 30 plots included for data analysis is noted in table 2. Litter depth data for each plot (the forty points sampled) was averaged to create a mean litter depth for each plot. This data was then sorted by community type and graphically analyzed.



Figure 2: The average litter depth of each plot graphed to show variation in results for each community type.

Results

Table 1 illustrates the community type and average litter depth of each plot used for this research. The range of average litter depth for the 30 plots is 3.25 to 10.95 cm. This wide range is better depicted in table 3, which shows the average litter depth for each community type. Pitch Pine forests have the most litter, with an average depth of 8.58 cm and standard deviation of 1.94 cm. Pine-Oak forests have an average litter depth of 7.48 cm and standard deviation of 1.04 cm. Oak-Pine and Coastal Oak forests have comparable litter depths. Oak-Pine forests have an average litter depth of 4.81 cm and standard deviation of 0.82 cm while Coastal Oak forests have an average litter depth of 4.41 cm and standard deviation of 0.81 cm.

There was some variance in the average plot litter depths for each community type, which can be seen in table 3. Table 3 shows the standard deviation and variance for each community type. However, the trends are still evident even though litter depth data differed from plot to plot. Since all of the plot average litter depths are within two standard deviations of the average litter depth for the corresponding community, the



Figure 3: The already determined succession of the Long Island Pine Barrens.⁷

Plat Community Average Litter Stan								
# Type		Average Litter	Standard Deviation (cm)					
1	Pine-Oak	5 035	2 306					
2	Oak-Dine	5.955	2,802					
2	Oak-Pine	5.50	2.002					
3	Ditch Dine	5.5	4.200					
4	Pitch Pine	0.1875	4.208					
5	Oak-Pine	4.05	1.805					
9	Pitch Pine	8.65	3.215					
10	Coastal Oak	5	3.444					
11	Pine-Oak	7.7725	3.427					
13	Coastal Oak	5.325	1.808					
15	Pine-Oak	8.0125	3.644					
16	Pitch Pine	8.55	3.866					
17	Pitch Pine	10.95	5.28					
18	Coastal Oak	3.4625	1.73					
19	Coastal Oak	4.6	2.555					
23	Coastal Oak	5.475	2.019					
24	Oak-Pine	5.6	1.798					
25	Coastal Oak	3.675	1.799					
26	Coastal Oak	4.05	2.08					
27	Coastal Oak	5.075	1.913					
29	Oak-Pine	4.1625	2.11					
30	Oak-Pine	4.8375	2.888					
31	Coastal Oak	3.5875	1.836					
32	Coastal Oak	3.25	2.079					
33	Oak-Pine	5.3625	3.103					
34	Oak-Pine	5.9875	2.482					
36	Pine-Oak	8.2	2.933					
37	Oak-Pine	3.9875	2.111					
38	Oak-Pine	3.6875	1.873					
39	Coastal Oak	4.9625	3.331					
40	Oak-Pine	3.74	4.209					

Table 1: The community and average litter depth results of each plot. Average litter depth = average of the 40 measured litter depths.

variance in the data can be considered usual.

In figure 2, the average number of tree oak seedlings per plot was compared to the average number of pine seedlings. This data was then graphed by community type. Pitch Pine and Pine-Oak forests had similar results, with 15.75 and 18.75 tree oak seedlings per plot, respectively. Both had an average of less than one pine seedling per plot. Oak-Pine forests have outstanding regeneration with an average of 32.83 tree oak seedlings and 6.10 pine seedlings per plot. Coastal Oak forests have an average of 25.36 tree oak seedlings and less than one pine seedling per plot.

Discussion and Conclusion

By comparing the data in figure 1 to the forest succession diagram in figure 3, it is evident that litter depth is correlated with the transitions of forest succession. In the early stages of succession, Pitch Pine and Pine-Oak have a high average litter depth per plot whereas the later stages of succession, Coastal Oak and Oak-Pine, have lower litter depths. Figure 1 directly correlates to figure 3, the already determined succession of the Pine Barrens. This data can be considered statistically significant since the data for each community type is within two standard deviations of its corresponding mean or average. Even though there was slight variation in the data due to inaccuracy of measuring and other factors, the data collected is still usable.

Furthermore, this research demonstrates better regeneration in the later stages of succession. Figure 2 shows that Coastal Oak and Oak-Pine forests have a higher density of seedlings per plot than Pine-Oak and Pitch Pine forests. The likely explanation is that relatively shallow litter depth permits sunlight to directly reach the soil for better tree regeneration. In fact, the tree oaks most common to the Pine Barrens (Quercus alba, Quercus velutina, and Quercus coccinea) require light litter cover and full to partial sun for seedling establishment.^{7,8} This coincides with findings of a higher density of tree oaks in areas of reduced litter depth.

Similarly, pine (Pinus rigida) requires exposed mineral soil, i.e. absence of litter, and partial to full sun for seedling growth. This is also reflected in the results since pine seedlings were only found in areas of light litter. However, it should also be noted that pitch pine cones can require exposure to fire in order to spread the pine seeds for growth. After a period of 10 to 20 years without occurrence of fire, the oak canopies close, restricting the soil's access to sunlight. The pine trees in the canopy can persist, but pine seedlings cannot germinate with the excessive litter and lack of sunlight.⁷

Furthermore, community transitions because of succession occur at a very slow rate without initiation by fire. Therefore, it is sometimes necessary to prescribe forest fires and establish and maintain them safely and correctly. In the near future, researchers should use the data findings of the Central Pine Barrens Monitoring Program to determine a litter depth threshold. This would enable prescribed fires to be properly timed for maximum conservation efforts. Restoration and management of the Pine Barrens should be established and started as soon as possible so that future generations can enjoy the unique and fascinating resources that it holds.

Acknowledgements

This research was conducted at Brookhaven National Laboratory with support from the Foundation of Ecological Research in the Northeast, the Central Pine barrens Commission, Long Island Community Foundation, and Brookhaven Science Associates. I would like to thank my mentor Timothy Green and my advisor Robert Anderson for all of their direction and guidance during the course of the summer. Additionally, I thank the U.S. Department of Energy, Office of Science, and the SULI program for allowing me the opportunity to participate in such an exceptional and fulfilling

Plant	Number of		Pitch Pine	Pine-Oak	Oak-Pine	Coastal Oal
Community	plots	Litter Depth (cm)	8.5844	7.48	4.8086	4.4057
Pitch Pine	4	Standard Deviation	1.9448	1.0448	0.8151	0.8181
Pine-Oak	4	Number of trees per community				
Oak-Pine	11	used in the study	4	4	11	11
Coastal Oak	11					

Figure 2: The community type breakdown of the 30 plots used in this research.

Figure 3: The average litter depth of each community type (the mean of the average litter depth for each plot by community type). Standard deviation and variance of each mean is also displayed.

internship program.

Special thanks also go to the other members of the program crew: Kathryn Gutleber, Andrew Siefert, Matthew Kull, Miranda Davis, and Chauncey Leahy. jur: Any advice you can give to fellow undergraduates who would like to do this kind of research? (or any other type of research)

My advice is to apply to summer undergraduate research programs. This way you can work with a mentor who will guide you through the research process while also allowing you the opportunity to complete your own research. I benefited tremendously from working with Dr. Tim Green. I hope to complete research in another area of biology this summer.

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About the Author

Dana Tievsky is currently finishing a B.A. in Biology as well as a B.A. in Psychology at the University of Rochester. She plans to attend dental school following her graduation in May 2007. This article is a slight adaptation of a paper written under Dr. Timothy Green of Brookhaven National Laboratory for the Department of Energy Science Undergraduate Laboratory Internship.

jur: How did you become interested in this area of research?

After completing biology coursework in animal behavior, I developed a strong interest in ecology. As a result I decided to focus my internship on environmental science. I was particularly drawn to this research program because I wanted to the opportunity to work with a mentor who would provide me he opportunity to learn more about research areas unfamiliar to me.

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

I am focusing my B.A. in Biology on ecology and evolution so this research directly relates to my studies with my major.

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

The biggest obstacle during my field research was getting used to the conditions of the Long Island Pine Barrens. Since there are lots of ticks, long pants and long sleeves were necessary. Learning to work in the heat of the summer with the appropriate "tick gear" was difficult to get used to.

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

I was honored when I was invited to discuss my research at the annual Pine Barrens Research Forum in Long island this October. Unfortunately, I was unable to attend the conference, but my poster and research were presented in my absence.



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About the Journal



The Journal of Undergraduate Research (ISSN# 1547*9641), Volume 4 Issue 2, Spring 2006 was assembled on an Apple PowerMac G4 Quicksilver using Adobe InDesign CS. Graphics were produced using Adobe Photoshop 7.0 and Adobe Illustrator 10. Microsoft Office was used for text editing and review. Fonts used included Adobe Garamond, Futura, Times New Roman, and Symbol (for Greek Characters). This 46-page journal was printed and perfectly bound by Mercury Print Productions Inc. of Rochester, NY.





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