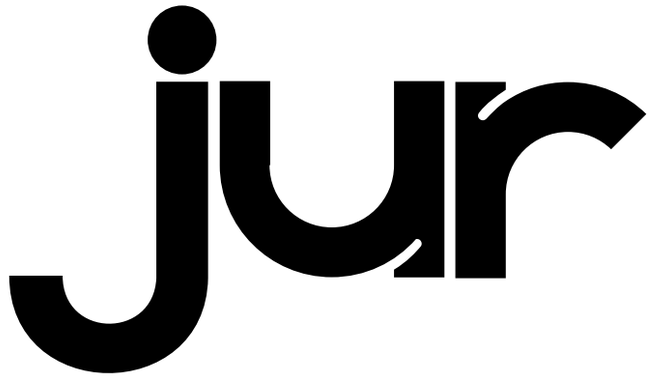
Journal of Undergraduate Research



University of Rochester

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

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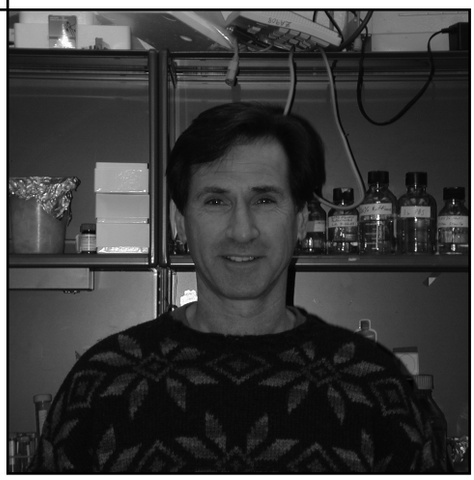
From the Editors

Great minds have over and over again expressed a belief that the best thinking and research anyone will produce is done before the age of twenty-five. Whatever that spark, that vitality is that we possess as young people, it drives us to ever-greater heights as we seek our place in the real world we are finally beginning to join. Our paths are myriad, our purposes are different, and yet success on every one of life's paths is predicated on one thing: good, precise thought. Whether our aims are for the business world, academia, politics, humanitarianism, or any other possible field, how far we go is limited primarily by how well we can teach ourselves to think. Thirty years from now, it will probably be the ability to think critically that all of us will have retained from our time here at Rochester, much more than any specific knowledge from your coursework. College teaches undergraduates like us how to think, and there is no better way to practice that art than by engaging in the most rigorous training for critical thought there is: research.

Research is challenging, exhausting, rewarding, illuminating, frustrating, endless, and countless other things all at once. But most of all, it is a life skill that will allow us to make our voices heard and see the application of ideals we believe to be right. All of it begins now, when the opportunity glistens before us to produce that research so many of our elders herald as the best we will ever do. For all of these reasons, here at *jur* we vehemently believe that undergraduate research is valuable both to the researcher who produces it and to the larger community, which is why we continue to put forth this journal. We see how often work is disregarded simply because it is produced by an undergraduate, and it is our hope that as *jur*'s circulation and recognition continues to increase, so with it will the recognition of the excellent research done not only by Rochester undergraduates, but by undergraduates everywhere.

Sincerely,

Sam Boyer, 2006 Jason Moore, 2005
Editors-in-Chief



Perspectives on Research

jur interviews David Goldfarb, Ph.D.

Dr. David Goldfarb, Professor in the Biology Department and a respected cell biologist discusses some of the oft-ignored, nontraditional aspects of a career in basic research, and the role of undergraduates in his laboratory.

jur: What kind of research are you involved with?

Goldfarb: I lead a group of molecular cell biologists. We are cellular mechanics who study the thousands of different parts in a cell and how they work in concert to facilitate various processes like energy production, motility, and replication. Most of the processes we study are fundamental—they occur in all animal and plant cells—but are somewhat esoteric and virtually unknown to non-aficionados. These processes are more complex than a series of linked chemical reactions, which might involve the concerted action of several proteins. The functioning of an automobile engine is an example of a cell-like process: the engine makes the car go. What makes cells move? Thousands of papers have been published on this one question. Like an engine that contains many parts, most cellular processes involve a large number of individual parts, most of which are proteins. Some of these proteins are structural, like a chassis. Other parts are functional, like pistons, and still others are regulatory, like gas and brake pedals. Processes also occur between different compartments in the cell, by analogy to the passenger compartment and under the hood. But cellular parts are very small—about 100 million times smaller than an engine—and are mixed together in an aqueous solution with thousands of other parts. Most proteins are exceedingly delicate, so they are easily damaged during experiments. Many proteins have lifetimes on the order of minutes or hours before they are degraded and replaced. Cells are dynamic entities. The cell body is continuously turning over and renewing itself. I think cells are better viewed as shifting patterns, like a waterfall, rather than static objects, like an engine. This, I think, is a beautiful concept: the cell is a paradigm of impermanence.

A typical task for cell biologists is to identify and catalogue all the parts that contribute to a particular process. While many of our working hours are spent identifying and characterizing the parts, we also enjoy guessing how the entire process might work. Processes begin as black boxes sometimes we have but a few pieces of the jigsaw puzzle to guess from. The job is analogous to giving a group of 18th century mechanical engineers the fuel injector and catalytic converter from a late model car and asking them to explain their function and the nature of the machine they belong to. Needless to say, I am really impressed with people who claim to be able to reconstruct an entire dinosaur from a single neck bone or tooth. So, you might imagine how satisfying it must be to figure out anything at all about cells. Some of the motivation comes from the fact that the questions we ask need only be solved once in the history of science, or at most a few times if different labs are working independently on the same project. It is rewarding to be the first person who discovers even the most trivial fact of life.

I will admit to being something of a mystic. I believe that the cells we study harbor mind-boggling secrets; if you read the journals you know this to be true. Moreover, every new fact induces a subtle shift in our conceptual landscape, changing our perception of the cell and ourselves. This is a process as slow as molasses, but it flows inexorably forward. This is why biology is a nice field to grow old in. Perspective helps.

jur: Aside from the personal rewards that come from solving these extremely difficult problems, why is it important to study cell biology?

Goldfarb: The 'biomedical' answer is what my parents and relatives want me to give them when they ask, "What is it good for?" Over the years our research has been funded by agencies like the American Cancer Society, March of Dimes, and National Institutes of Health, whose missions are to prevent or cure human suffering. Lucky for me, these agencies appreciate that gaining a basic understanding of how healthy cells and bodies work is an important part of reaching these goals. Any (good) automobile mechanic knows it's next to impossible to diagnose and fix a sick engine if you don't know how the darn thing is supposed to work when it's healthy. This is not to deny the utility of time-tested empirical approaches to both medicine and mechanics: "Eat this root, it helped me," or "Kick the tire and bang on the hood." But I don't know of any other way than the scientific method to elucidate cell mechanisms. There is no empirical approach to understanding vesicular dynamics. A strong suit of science is its calloused willingness to destroy our most beautiful and cherished ideas. The physicist Richard

Feynman said, "Science is the art of not fooling yourself."

So, the study of basic cell processes is important because it benefits the work of biomedical and medical researchers and physicians. Although cell biology is partly a game or just a career for some people, it is ultimately a compassionate endeavor, at least in my opinion. We can track these benefits by using a web-based tool that displays all the publications that reference our papers; such citation records provide interesting insight into the 'life' of a result. Back in 1993 we published a paper with another group that explained how HIV-1 gets into the nucleus to do its dirty work. As of this week, the most recent of 454 papers to cite the HIV-1 study is titled, "Lentivirus-mediated gene transfer to the respiratory epithelium: a promising approach to gene therapy of cystic fibrosis." Who knew? Another of our papers also published in 1993 has been cited 154 times, but only by other basic research papers. A 1997 paper on the evolutionary origin of certain proteins—it doesn't get more basic than this—has been cited 60 times, most recently by a paper titled, "Nuclear transport and cancer: From mechanism to intervention." So it goes.

There's something else to say about the HIV-1 paper, though. Our contribution to that paper was technically and conceptually relatively trivial compared to our core research efforts, but the result attracted radio and television attention. I appeared on the 10 o'clock news and was interviewed on local National Public Radio. One caller challenged me to defend the charge that the US government was using the AIDS virus to attack African American communities. I wish I had been better prepared. The important point here is that this 'trivial' but relevant result could not have been discovered had not we and many others been sequestered away for years with our little tubes and pipettes, like monks in their monasteries performing esoteric rituals. Behind every headline acclaiming this or that cure are myriad studies on cellular phenomena about which few non-experts could even begin to understand. There are no shortcuts. We have got to keep stoking the engines of basic research. This is something I feel that our current administration may not fully appreciate.

jur: You have recently discovered a new form of autophagy in yeast. What is autophagy, and why was your discovery significant?

Goldfarb: A few years ago we discovered a new form of autophagy ("self-eating"). This was very exciting. I told everybody I knew. We call this process "piecemeal microautophagy of the nucleus" (PMN). Catchy, eh? In this process, pieces of the nucleus are pinched off into an organelle called the vacuole, where the pieces are broken down into their constituent parts and recycled back to the cell. Autophagy has a lot to do with recycling broken, damaged, or useless cell parts. The nucleus had not been considered a substrate for autophagy because it is an essential organelle – lose your nucleus and you die. But PMN circumvents this problem by removing small, unimportant bits of the nucleus. Best of all, PMN is a beautiful process that can be observed in real time under the microscope using fluorescent tags. PMN occurs at accelerated rates in starving cells, when a lot of the stuff found in growing nuclei are not needed, and could be put to better use elsewhere. By analogy, people will burn tables and chairs when there is no other way to keep warm. Burning tables and chairs and degrading pieces of the nucleus are both ways to get through tough times.

Before PMN came into our lives, we focused on nuclear transport. Nuclear transport is the process that facilitates the import and export across the nuclear envelope of proteins and RNAs, and during infections, virus particles. Recently we have been concentrating on the molecular mechanism of the nuclear pore complex (NPC). This machine is a transporter with fantastic properties. The NPC resembles the type of space station visionaries imagine will someday orbit the earth. Instead of orbiting the planet, the NPC is embedded in the nuclear envelope. We know all the protein components of the NPC, but neither how they fit together nor how the machine works. The molecular mechanism of the NPC is such a difficult problem that a number of my colleagues have abandoned it for richer pastures. Since the consistency of the central channel of the NPC is something like a jelly donut, the thing loses its shape and falls apart when you try and isolate it.

My group specializes in studying processes as they function undisturbed in cells. We use assays that measure rates of transport through the NPC in living cells. There are a lot of tricks involved. Recently, these experiments led us to conclude that there are different routes across the NPC. This was a surprise, since most cell biologists had guessed that one pathway would have been enough. There is a lot of biomedical interest in nuclear transport because it is intimately involved in gene expression during growth, development, and disease.

Lately we have been excited about a new project to identify genes that control lifespan and aging. This is a field we are going to be hearing exciting things from in the near future. We have a novel approach and a clever assay. In real estate, "It's location, location, location." In my field, "It's the assay stupid." Keep an eye out.

jur: You focus a lot of your research on yeast. Why does yeast serve as a good model for human cells, and what are the advantages of using yeast?

Goldfarb: My group now works mostly with the brewer's or budding yeast *Saccharomyces cerevisiae*. Yeast is a eukaryote like plants and animals. The processes we study in yeast also occur in human cells. Some of the parts are indistinguishable, and many more are very similar. People have taken human proteins that play key roles in cell replication and expressed them in yeast. Remarkably, some of these can substitute for their yeast "homologues." I am pretty sure most people don't appreciate how similar we are to yeasts, or to any other cells on the planet for that matter. A remarkable degree of what we know about human cells has come from studying yeast.

I work with yeast not only because they provide us with a powerful and facile experimental system, but also because I choose not to work with vertebrate animals. This is a personal choice. I don't have feelings towards my well-meaning colleagues who work with vertebrates, although I can't understand how anybody can do experiments on primates. I appreciate the difficulties inherent in this rationale, and I do not claim to have an answer to the question which I sometimes ask myself, "What if my child gets a horrible disease that could only be cured by animal research?" Perhaps my choice is conditional on not having to answer that question. For now, I am just

happy that my group can do interesting work without harming (many) laboratory animals. We stopped using frogs, mice, rats, and rabbits over a decade ago. We had a holdover from those years, a pet *Xenopus laevis* frog. She died a few weeks ago at the ripe old age of 13 years, and she has been replaced with one we rescued from the experimental tanks on the third floor.

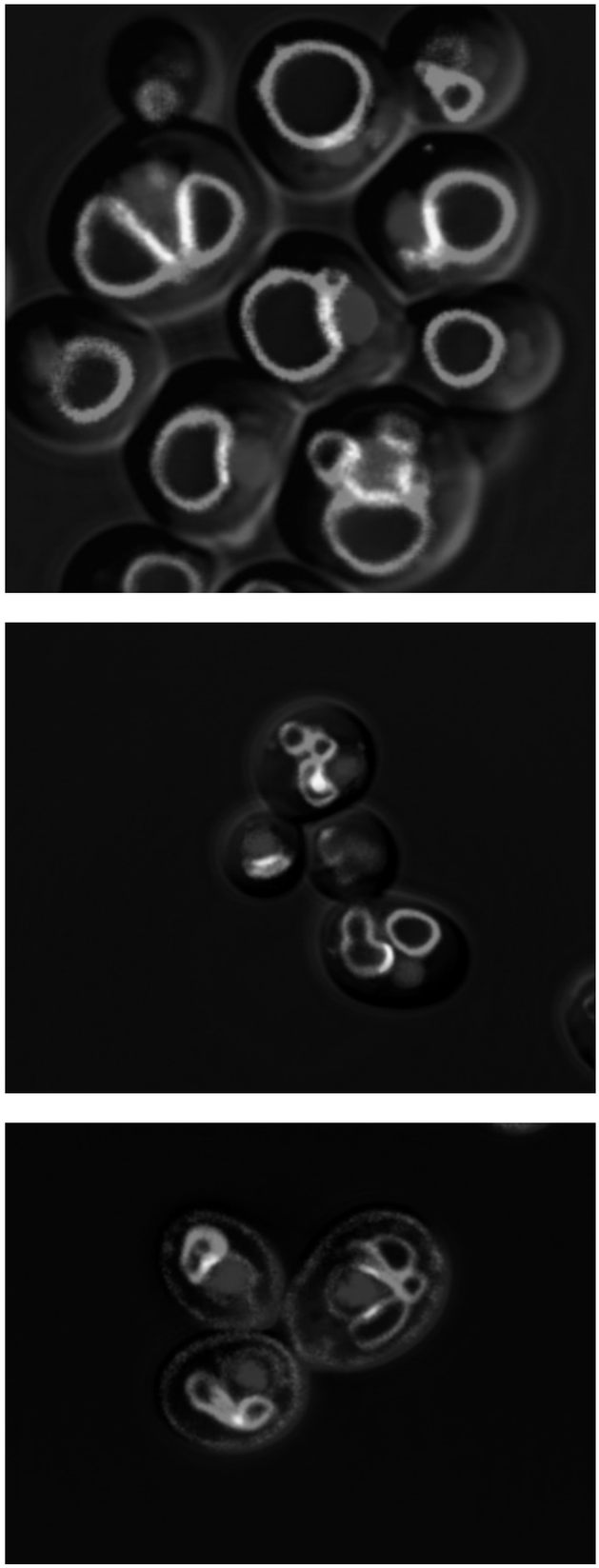
My general guideline is to avoid experimenting on animals with nervous systems. It used to be "animals with faces," but I thought that made a mockery of a serious situation. Recently, a student in my lab had good reason to work with fruit flies, so we ended up killing lots of them. I can rationalize using flies, even though I know they have lives too. One recurring problem is that we can't get all of our work done without using antibodies raised in rabbits (antibodies are sometimes raised in hamsters, mice, chickens and even goats). These animals suffer and ultimately die during the production of antibodies, so I am mindful of where our antibodies come from and try to use them judiciously. So I do break the rule. There is a disconcerting amount of blood, sweat, and tears in research aimed at reducing suffering.

jur: Was there some particular event that made you decide to stop using animals in your research?

Goldfarb: The decision to stop using vertebrates in the lab was influenced by events long ago. My very first day in a research lab as a freshman at the University of San Diego included watching the beheading, with a nifty miniature guillotine, of twenty or thirty mice. The objective was to harvest fresh pituitary glands, which were plopped unceremoniously into liquid nitrogen. I still remember the odor associated with the slaughter, and the memory still evokes the repulsion I felt when I saw their heads gasp for air. I also remember being ashamed at being repulsed. Later, during my senior year, I made a huge gaffe and accidentally put 200 mice in rat cages. The bars were too widely spaced to contain the mice. During the night many of the mice escaped their cages and had a big party in the animal facility. By morning there was no way to tell which mice belonged to which group. The project was ruined, and it fell to me to euthanize all 200 mice by CO_2 asphyxiation. This episode affected me deeply. For this and other reasons (including lessons learned from shooting at animals and the influence of positive relationships with cats, dogs, and horses) I no longer eat anything with a nervous system.

jur: Since your interaction with undergraduate research tends to be through students in your lab, could you tell us about undergraduate research in the laboratory context?

Goldfarb: There are a number of good reasons for undergraduates to work in a real research lab. One of the good reasons for coming to the U of R is the opportunity to experience world-class research firsthand. Research is how we learn in the sciences and it's also useful in the arts. Lab courses are one means we offer students to experience lab work, but some wish to go further. Even a brief apprenticeship in a lab can be a transforming experience. For some, it becomes clear after a few weeks that they are either not cut out for, or not interested in research. This is good to know. Others discover a love of the work and never look back.



The guts of a cell. The machinations of living *Saccharomyces cerevisiae* ("brewer's yeast") cells can be observed in real time using fluorescent stains visible under the confocal microscope. These figures depict the regulated degradation of the nucleus and the formation of junctions between the nuclear membrane and the vacuole.

I have no hard and fast rules about who gets to come into my lab. In my junior year at UCSD I was interviewed by Jonathan Singer for a position in his lab. Dr. Singer is famous for the fluid mosaic model of biological membranes. I remember him looking directly at me from across his vast desk and pronouncing that he saw nothing exceptional in my record. There was no place for me in his lab. That was a healthy dose of reality. Instead of climbing back down my hole, I joined the lab of Katsumi Miyai, who was less judgmental, but still a very serious scientist. I published my first paper in his lab. Now that I am the one sitting behind the desk, I don't always look for something exceptional. Still, the students I invite into the lab usually do impress me with some personal quality, such as motivation, excellence in areas outside of school, or simply an engaging personality. However, there has to be evidence of an innate seriousness. Paradoxically, a goofy student can still be dead serious. Taking students with good grades—at least a strong 'B' average—is important for two reasons. First, there is a correlation between performance in coursework and performance in the lab, and it is fair to give students who have a future in research a chance. Second, students who are struggling with their classes usually cannot afford the commitment needed to work in a lab. However, there are exceptions; in fact I was an exception. Paul Saltman at UCSD invited me to work in his lab at the end of my freshman year because I was struggling with my coursework. His lab, which was a very supportive environment, helped me to gain a lot of confidence. With this new measure of confidence I found myself better able to invest in studying, since there was now hope for a good outcome. Because of this experience I periodically accept students who are struggling with their coursework. I have read or heard a lot of like-minded parables about horses or calligraphers or sons or daughters that attest to the deeper understanding or greater accomplishments of those who struggle in the beginning. Being truly gifted is not a ticket to success in research. Analytical skills never hurt, but they are neither sufficient nor necessary. Intangibles like showing up to work with eyes and ears open are the aces up many students' sleeves. I have come to believe that a person's unique personality is just as important in research as their intelligence and motivation.

jur: What are some of the obstacles that you face in your lab, and how do these affect undergraduates in particular?

Goldfarb: Students who enter the lab soon discover that it is not for the faint of heart. My lab has always been a pretty intense place; we are not fooling around. Those who enter the lab with the sole intention of adding to their resume or getting a recommendation rarely last very long. The work has to be intrinsically important to the student, which is something that cannot be manufactured. Persistence in the lab is a self-selecting trait. When things are not going well I might remind the student that "we are struggling with the beast." By this I refer to the sensation that our yeast cells almost maliciously resist giving up their secrets, that they are designed by a greater power to be recalcitrant. Cells are extremely subtle entities. We can't directly experience the inner workings of cells, so we need to develop sophisticated and imperfectly indirect methods. You should see some of the microscopes we use these days. We also develop our own cell-based assays. There are no kits for what we do. We usually start using these assays before the kinks have been removed, and sometimes even before we have the correct equipment for the job. There is no time to wait, since we really need to know the answer. Bailing out a boat with a sieve can be done, but it takes commitment.

I think it is also very important to note that having undergraduate researchers in my lab is great for me, even though undergraduates sometimes have trouble accepting how often, repeatedly, and miserably we fail. I have seen many students who couldn't deal with this reality. The quality of falling down, picking oneself up, falling down again, and picking oneself up again is a regular experience for most people, at least in my lab. I believe this is the experience of anyone who strives to achieve excellence in any field. Many of our top undergraduates sail through their coursework and expect their independent research projects to be the same. Sometimes they are. Sometimes the project and the student just click and nothing could be easier. This is rare. But everyone eventually hits some kind of wall, and often they hit it hard. It takes a strong ego-structure to survive in the lab long-term. Maybe students are ultimately attracted to independent research because it feels real, it is real. An extremely gifted teacher of mine once signed a book of his over to me with the lines, "If not you, who? If not now, when?"

Further Reading List:

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Featured Researcher

Performance During Visual Search:

Designing Spacecraft with Humans in Mind

Joseph Toscano, 2005

Advised by Brent Beutter, Ph.D.
National Aeronautics and Space Administration

This Featured Researcher article focuses on Joseph Toscano, a senior majoring in Brain and Cognitive Sciences, who did his research at the National Aeronautics and Space Administration (NASA) under the guidance of Brent Beutter. Joe plans to attend graduate school in cognitive science after graduation.

What is visual research all about?

Visual perception is the process by which the brain interprets the visual information that enters our eyes. How does the brain turn waves of electromagnetic radiation into objects we can recognize, such as a friend's face, a coffee cup, or the cockpit in a Space Shuttle? We can study this process by using an eye tracker to record a person's eye movements as they look at a scene in front of them. Eye movements are closely linked to attention, and they allow us to see what someone is thinking about, based on where they are looking, at any given time.

Attention is an important part of perception, since the brain can only process a limited amount of information at once. By studying eye movements in a controlled condition, we can understand how the brain processes visual information and allows us to perceive the world as we do.

Who or what motivated you to start on this research project?

My research was done through the NASA Undergraduate Student Research Program (USRP). This is an internship program for undergraduate science and engineering majors that allows them to do research at one of several NASA facilities. I first found out about the program from two of the graduate students in our lab, Ellen Campana and Kate Pirog. I had always wanted to work for NASA, and this internship gave me that opportunity. My adviser at NASA Ames, Brent Beutter, was essential for motivating me to design an experiment to study visual attention.

How does it relate to your major?

BCS (Brain and Cognitive Science) is a very extensive major, and it includes many different subjects relating to how the brain functions. Some of these topics are language comprehension, decision making, attention, memory, and perception, including visual perception. Studying vision is an important part of understanding how all the parts of the brain work together to allow us to perceive the world. Also, the research I did at NASA is closely tied to human factors, which is the study of making machines that are well-suited to the cognitive capabilities of human users.

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

The most fulfilling experience was being able to say that I've worked for NASA. I mean, how many people can say that? More importantly though, the research I did this summer has helped guide me towards what areas of cognitive science I would like to study in graduate school. The internship this summer also helped to solidify my desire to go into research, and I am grateful I had the opportunity to participate in the USRP.

How can undergraduates get started in this kind of research?

To apply for the NASA USRP, go to the NASA Education website at http://education.nasa.gov/. From there, you will find links to the USRP site. It's also very easy to get involved with research here at UR. Read about the research being done in your professors' labs, and if it interests you, contact them and tell them that you are interested in being a research assistant. Faculty in the BCS department are conducting research on a wide array of topics, and many of them like to have undergraduates working with them.

Figure 1: The cockpit of the Space Shuttle Atlantis while in orbit around Earth on mission STS-101. This image shows the new touchscreen panels and displays that were installed.

We use our visual system to continuously update our information about the world around us. By looking at various objects in the environment, we acquire knowledge about the properties of the objects and their locations. One important aspect of this process is visual search—the ability to locate and identify objects in a complex, noisy environment. Visual search allows us to routinely analyze the world and gain information about it. One situation in which visual search plays a significant role is in space. The ability of astronauts to successfully search for objects in a complex environment, such as a spacecraft cockpit, is crucial for safe manned spaceflight. Understanding how humans perform visual searches allows us to design interfaces that are well-matched to the capabilities of human users.

When performing a visual search task, the brain signals the eyes to make rapid movements, known as saccades, to various places in order to gain information about the objects present. The mechanisms that control the location of eye movements are determined by the task, the observer's biases, and the properties of the objects present in the scene. Patterns of eye movements are particularly important in visual search tasks in which the observer must scan a display to locate a target.

Eye movements are closely linked to attention. While it is possible to attend to a location without making a saccade to it,[1] the converse situation is not necessarily true.[2,3] Slightly before a saccade occurs, attention normally shifts to the location of the eye movement. Thus, eye movements provide us with a good observable phenomenon to test where an observer's attention is focused. Since eye movements and attention are so closely linked, studying eye movement patterns help us gain an understanding of where an individual's attention is focused in a complex environment, such as the Space Shuttle cockpit (Fig. 1).

Several parameters affect when and where eye movements will occur. One set of factors, often referred to as bottom-up factors, consists of the properties of the stimulus itself, and are determined by low-level mechanisms in the brain.[4-7] These factors include contrast, color, shape, onset, location, and a number of other features. Stimulus features give us a measure of an object's salience, that is, how well it "stands out" in a scene. The other set of factors, top-down factors, include the observer's conscious decisions about where to focus his eyes, and are determined by higher order processing and decision making areas in the brain.[8] While we often make eye movements without conscious thought, it is also possible to exert a great deal of conscious control over where we are looking. If, in a visual search task, an observer is looking for any objects in the scene that have a certain set of features, a purely top-down system would be able to attend to only objects that exhibit those features and ignore those that do not.

Understanding how the brain controls these processes is important for knowing how we should design spacecraft controls to minimize the mental workload that astronauts must cope with. For example, a crucial aspect of a Shuttle mission is lift-off. While this procedure is largely computer controlled, the crew must act quickly if an emergency situation arises. The Space Shuttle cockpit controls consist of a mix of brightly lit flat panel displays, as well as numerous switches and buttons that are not illuminated. In this environment, is an astronaut's attention guided by the properties of the controls themselves, that is, bottom-up factors, or is the focus of attention determined by the astronaut's objectives and goals, that is, top-down factors? Neither bottom-up nor top-down influences will exert complete control over eye movements. To what extent do these two sets of influences interact to determine the location of eye movements, and consequently, attention? This experiment was designed to answer these questions in a specific task condition that we can apply to the needs of astronauts in the operation of a spacecraft.

Bottom-up influence

If the task is dependent on mostly bottom-up factors, we expect to see an improvement in performance with an increase in target salience. In this experiment, the target and distractor items varied by contrast level. There are a number of possible ways contrast could affect performance. One possibility is that only the contrast of the target would be relevant to determining task performance. If target contrast is increased, there is an improvement in performance regardless of the level of the distractor contrast. Alternatively, performance may depend on both the contrast level of the target and the distractor. This relative salience hypothesis states that the

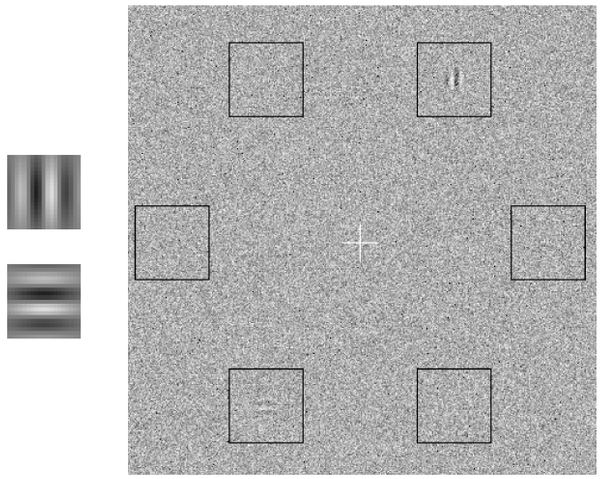


Figure 2 (left): Cue images used in the experiment.
Figure 3 (right): Sample stimulus image. Target (horizontal item) contrast at 12 percent; distractor (vertical item) contrast at 24 percent.

difference in contrast level between the two will determine the level of performance, not the target contrast alone.

Top-down influence

If top-down effects dominate in this task, we can expect that the observer's knowledge of the task and his or her goals will determine the location of saccades. If top-down control is used, the visual system would be acting as a filter, allowing objects that match the target to pass and discarding information about distractor items. Optimally, an observer would actively disregard information about the distractor instead of simply searching for objects matching the target item's properties. The top-down hypothesis predicts that performance would be determined by a top-down filter favoring the target items.

Methods

Previous research has shown that patterns of saccadic and perceptual decisions are similar.[9,10] It has also been established that an eye tracking paradigm is useful for studying visual search tasks.[11,12] In this experiment, an eye tracker was used to measure the observers' performance.

In this experiment, the observers were presented with a series of stimuli in which they were required to locate a target object. A cue image (Fig. 2), indicating which item represented the target, was presented at the beginning of the experiment. The stimuli consisted of horizontal and vertical grating patterns, called sine wave gabors, in the presence of noise. Each image contained one target item and one distractor item in two of six possible locations (the other locations contained only noise). The target and distractor items always differed in orientation by 90 degrees. That is, if the target item was a horizontal pattern, the distractor item was a vertical pattern. The target and distractor varied by contrast level, and each run was blocked by target contrast. Four contrast levels were used for the target and five for the distractor (including a "zero" level in which no distractor was present). Figure 3 represents a sample stimulus image. The target and distractor items were presented randomly, and all possible combinations of contrast level and orientation were used. There were 144 trials per run, and a series of four runs gave a complete set of data.

During each trial, observers were presented with a stimulus and were required to identify the location of the target in one of the six possible locations. They used a mouse to initiate the trial, chose which location they thought contained the target image, and then continued to the next trial. Observers used the left and right mouse buttons to rotate a pointer and pressed the middle button to indicate their response. In addition to the target, a distractor item was present in one of the other locations. A set of control trials that had no distractor were also included. The observers were told that the stimuli may contain a distractor, but they were not given explicit instructions to avoid looking at it. They were simply told to indicate which location contained the target image. On each trial, the observers' eye movements were recorded as well as their perceptual responses about the location of the target.

Two observers with normal or corrected vision participated in the experiment. After the experiment, each observer's results were analyzed. Only the observer's initial eye movement for each trial was measured.

Results

The results indicate a clear bottom-up effect on performance. The proportion of correct saccades increased with the relative salience of the target item (Fig. 4). Both an increase in target contrast and a decrease in distractor contrast

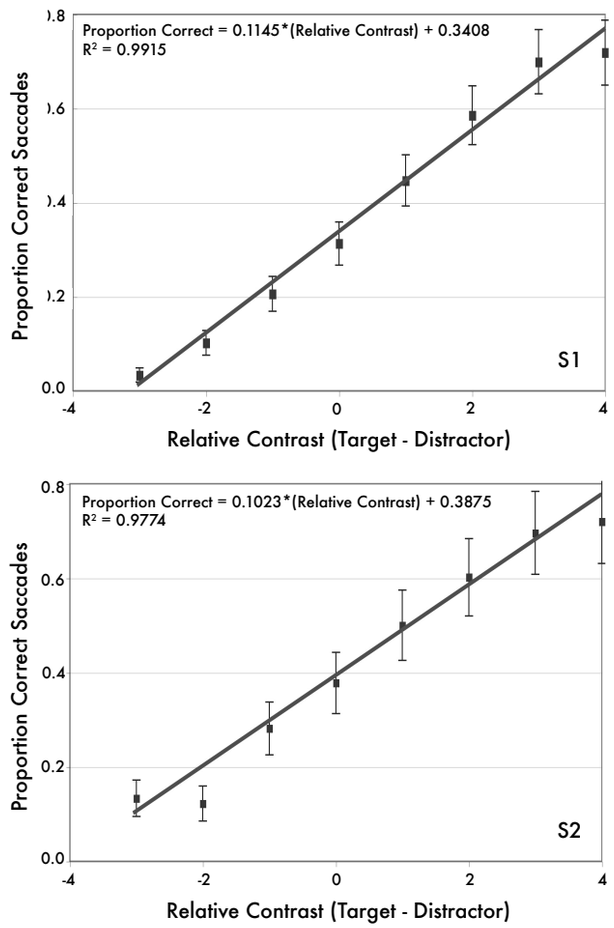
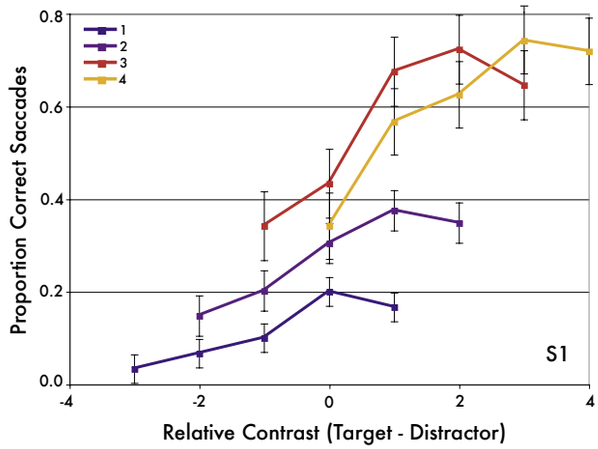


Figure 4: Proportion of correct initial saccades by the relative contrast difference between the target and distractor items for the two observers. Error bars indicate standard error. The line in each plot represents a linear fit of the data.



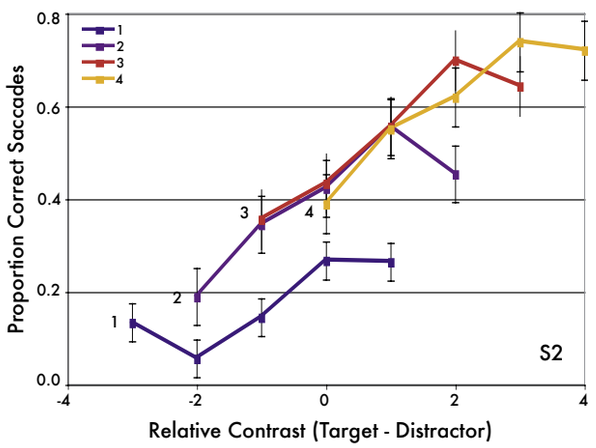
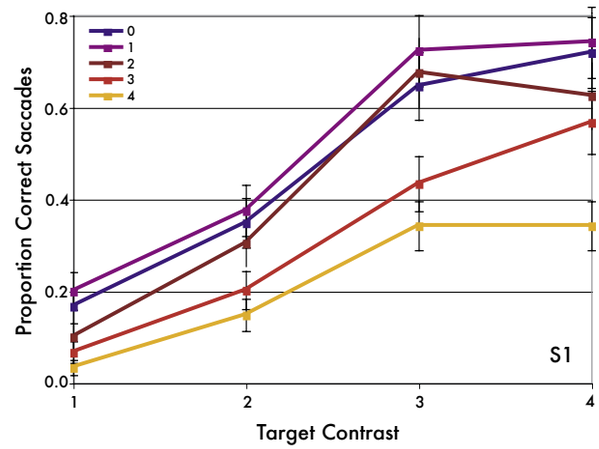


Figure 5: Proportion of correct saccades by relative contrast at each target contrast level presented for the two observers. Error bars indicate standard error.



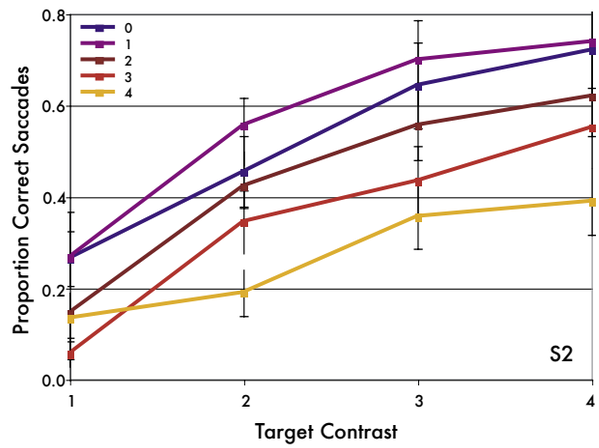


Figure 6: Proportion of correct saccades by the target contrast at each distractor contrast level for the two observers. The "0" line represents trials with no distractor item present. Error bars indicate standard error.present. Error bars indicate standard error.

improved performance. However, the relative difference does not account for all of the results. Figure 5 shows that there was a significant difference in performance for each of the target contrast levels. Also, there was an improvement in performance for a given target contrast when the distractor contrast was lower (Fig. 6). All of these results strongly favor the relative salience hypothesis.

Weak top-down effects were also present. These can be seen by looking at the proportion of eye movements to the distractor items. If relative salience determines performance, it is helpful to look at how often the observers looked at the distractor for each relative contrast level. Figure 7 is a plot of the proportion of saccades to each item by its own relative contrast (i.e. a contrast of "-3" for the target saccades corresponds to a "+3" condition for the distractor saccades). The mean proportion of saccades to the distractor was slightly lower than the proportion of saccades to the target for most of the relative contrast levels. This indicates that an item received more attention if it was a target than if it was a distractor.

Discussion

In the task presented in this experiment, bottom-up influences, namely the contrast of the two items in the display, determined the focus of the observers' attention. Even though the observers knew the orientation of the target item, they were still unable to initially disregard the irrelevant information about the distractor. Thus, the programming of eye movements by the brain, in this task, likely occurred primarily via low-level mechanisms rather than higher order processing based on the goals of the observer. It should be emphasized that this effect is task-dependent. Our perceptual results show that once the display has been adequately scanned and the observer has had the opportunity to examine each location, performance is nearly perfect (98-99 percent correct) with the exception of the lowest contrast level (65-70 percent correct). However, when initially viewed in the periphery of the visual field, it is possible that the orientation differences are less visible, and observers were forced to foveate each item in order to determine its orientation and make a perceptual decision.

The orientation of each item may not have been a distinct enough feature to allow for top-down control, causing the observers' knowledge about the task to be less useful. Also, it is possible that both the target and distractor were included in the attentional filter.[8] A future experiment that varied the target and distractor items by a different property, such as color, might produce results favoring top-down processing.

These results are consistent with theories that the programming of saccadic eye movements by the brain is regulated by two pathways; one receiving input from higher order brain areas and the other from low-level perceptual areas.[3,8,13] However, neither of these pathways solely determines

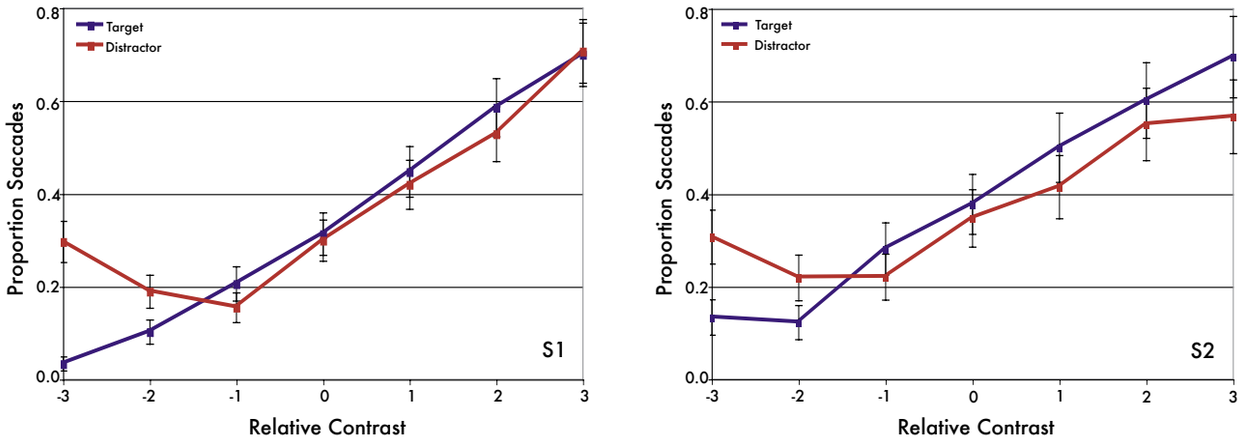


Figure 7: Proportion of saccades to the target and the distractor by relative contrast for the two observers. Error bars indicate standard error.

the pattern of eye movements that occur.

What does this mean for the problem of designing spacecraft controls? Since the level of attention an object receives is dependent on its salience relative to the other objects in the display, we must carefully choose which objects will have a high salience. For example, a brightly displayed altimeter will be easy to see, but it will also be extremely diffcult to ignore if our task is to locate the engine shut-off controls. While a high contrast object will serve as a good target, the same object will serve as an almost equally good distractor in a different task. Therefore, for a given task, the target of a visual search should not only be suffciently visible overall, but it should also have a high salience relative to other objects in the display. Ideally, the spacecraft's computer would dynamically change the contrast levels of the individual display elements so that they are optimal for the current task. These results are critical for NASA's design of manned spacecraft, including the Space Shuttle and the Crew Exploration Vehicle (CEV).

Acknowledgments

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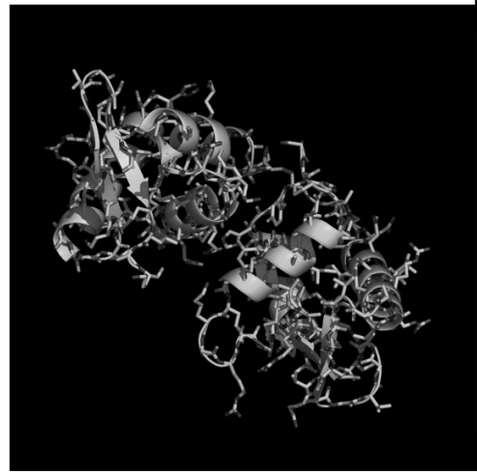
Solving the H1 Enigma:
The Use of *Physarum polycephalum* as a Model System



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The discovery of DNA as the molecular code of life has shaped the biological sciences for decades. However, with this knowledge, a question must be asked: how can the nearly two meters of DNA contained within the human nucleus fit into a compartment only 0.6×10^{-6} meters in diameter? Obviously, DNA must somehow be compacted, a task made difficult by the highly negatively charged phosphate backbone as well as the sheer length of each DNA molecule. The cell solves this problem by the formation of a highly condensed state of DNA termed chromatin. The primary unit of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped in 1.7 super-helical turns around an octamer of histone proteins H2A, H2B, H3 and H4.[1]

Each histone protein consists of a characteristic histone fold domain as well as relatively unstructured amino and carboxyl terminal tails. The histone fold domains serve as the site of protein-protein interactions and allow the eight sub-particles to combine into a disk-shaped unit.[1] The highly basic nature of the histone proteins allows formation of electrostatic bonds with the negatively charged DNA. Because the formation of these bonds is independent of the DNA sequence, the entire length of DNA is able to associate homogeneously within arrays of nucleosomes. This primary level of condensation gives rise to a 10 nm fiber or "beads on a string" conformation in which short lengths of linker DNA separate each nucleosome.[2,3] Obviously, several orders of further folding are required to achieve the compaction of DNA required by the cell. However, the mechanism of this compaction is rather poorly understood.

The association of DNA into nucleosomes and higher orders of folding vastly restricts the ability of enzymes to interact with the DNA. This means that in order for DNA replication or transcription to occur, the higher order structures must be resolved and the nucleosomes remodeled. It should also be noted that individual amino acids of the core histone tails are distinctively modified.[4] These modifications specifically alter the properties of DNA-nucleosome binding. For example, methylation of the lysine 4 residue of H3 has been correlated with active genes while H3 methylation at lysine 9 promotes gene silencing and heterochromatin formation.[5] Thus, specific modification of histone tails has interesting applications in gene control, as these modifications can determine the transcriptional fate at specific loci.

Functional Role of the Linker Histone H1

In addition to the core nucleosome, the linker histone (H1) is also a major part of chromatin. Association of H1 with the nucleosome protects an additional twenty base pairs of linker DNA.[6] Composed of a short amino terminal domain, a globular domain, and a longer carboxyl terminus, the H1 protein is also highly basic in nature, but does not share the histone fold domain present in the core histones. H1 bends DNA entering and exiting the nucleosome and has long been believed to stabilize condensed chromatin.[6] However, due to the difficulty of crystallizing H1 in association with core histones and DNA, several different models have been proposed to explain the interaction of H1 in chromatin. Traditionally, in vitro studies have implicated the linker histone in affecting chromatin structure several ways, including the stabilization of DNA entering the nucleosome core, restriction of nucleosome mobility and spacing and stabilization of higher order structures.[7-9] These characteristics would place H1 in a position to globally restrict gene transcription. However, it has additionally been shown that these outcomes can be partially or completely mimicked by increased salt concentrations. Thus, a question remains: what role is H1 performing inside the cell?

The best way to begin answering this question is to perform *in vivo* experiments in which the gene encoding for H1 is removed or mutated and the effect on the organism is observed. Because the overwhelming majority of biological mechanisms are conserved from yeast through humans, it is useful to perform these studies on lower organisms as this allows simplification of the experiments and data interpretation.

Studies performed in yeast involving H1 knockouts showed that the absence of H1 did not significantly perturb chromatin structure or viability, meaning the organism was able to successfully complete mitosis and transcribe the required genes.[10] This result was replicated in *A. immersus* and *Tetrahymena*.[11,12] Furthermore, in lower eukaryotes studied thus far few phenotypic changes have been observed. It is interesting to note, however, that H1 has been recently implicated in aging and genome stability as yeast and *A. immersus* show shorter life spans when H1 is not present.[11,13] The findings in lower organisms further complicate our ideas about the role of H1 in the cell. If the protein is not essential in lower organisms, why would it be conserved through humans? It is extremely

unlikely that a protein serving no function would withstand evolutionary pressures. However, because both the yeast and *Tetrahymena* H1 protein structures deviate from the common tripartite structure (yeast having two globular domains and *Tetrahymena* lacking a globular domain) the data from these organisms must be carefully analyzed.[14]

Another clue into the mysteries of H1 comes from null mutations in mice. Mice, like the majority of higher eukaryotes, contain several variants of H1. When researchers created null mutations of one subtype, no phenotypic difference was seen.[15] However, these animals retained normal H1-to-core histone ratios thus suggesting that other H1 subtypes were compensating for the missing protein. To test this theory, researchers generated compound null mutation mice in which three H1 subtypes were absent. These mice were not viable and showed a wide range of phenotypic abnormalities which resulted in embryonic death, thus proving H1 is indeed essential in mice.[15] However, these results give little indication of the actual function or mechanism of the linker histone.

Because knockout studies of H1 have shown little effect on global transcription, perhaps H1 provides a more specific function within the cell. Gorovsky examined the effect on individual genes in *Tetrahymena* and found that basal transcription of some repressed genes including (ngoA) was increased while the transcription of activated genes (CyP1) was decreased.[16] This surprising result indicates that the *in vivo* effects of H1 are indeed gene specific and may involve either up or down regulation of the transcribed gene.

Further evidence for a specific, tightly regulated function for linker histones has been shown in mice. The linker histone subtype H1b has been shown to bind in conjunction with the protein Msx1 at the regulatory element of MyoD.[17] The MyoD gene encodes a protein that controls the differentiation of skeletal muscle. When both H1b and Msx1 bind, the MyoD locus is repressed, therefore the MyoD gene is not transcribed and muscle differentiation is inhibited in cell culture.[17,18]

The role of H1 has also been extended to include the control of DNA repair by homologous recombination. Inhibition of the yeast H1 homologue Hho1p shows no significant phenotypic alteration when the protein was knocked out from yeast cells. However, the mutant strain was able to survive high levels of the DNA damaging agent methyl-methane sulfonate (MMS).[13] Through a combination of knockout studies, it was shown that disruption of Hho1p increases survival in the presence of MMS only when the genes responsible for homologous recombination were available.[13] Therefore, the presence of the linker histone Hho1p inhibits DNA repair by homologous recombination, which results in a reduced life span of the organism.[13] While it is possible that the regulation of homologous repair by H1 is due to DNA condensing properties, this explanation seems unlikely as knockouts of Hho1p do not exhibit a noticeable change in chromatin condensation.[13] Rather, it is possible that the presence of a linker histone covers binding sites for homologous repair machinery. If these results are conserved through humans, H1 regulation may have important consequences in cancer biology as mutation of H1 could promote tumorigenesis through enhancing rates of homologous repair.

Linker histones have also been implicated in the inhibition of ATP-dependent chromatin remodeling activities. Phosphorylation of linker histones *in vitro* inhibited the activity of several ATP dependent chromatin remodeling complexes including ySWI/SNF, hSWI/SNF, xMi-2, and cACF.[19] This activity was shown to be independent of H1's ability to condense chromatin as the same inhibition levels were observed following modification of core histone tails. This modification prevents the DNA from folding into higher order chromatin structures. Furthermore, it was shown that the inhibitory action of linker histones on ATP dependent remodeling complexes could be countered by introducing a kinase to phosphorylate the linker histone.[19] This suggests a tightly regulated pathway must be involved in the modification of linker histones at specific gene loci.

The results presented above provide a clearer indication of the role of H1 in regulating specific gene transcription, yet one is left asking what actually allows these functions to occur. H1 binds indiscriminately to DNA, yet this does not explain the specific, regulated functions observed in these studies. Perhaps, similar to the core histones, the linker histone is somehow modified in order to bring about these specific regulatory functions. It seems the linker histone story is much more complicated than simply stabilizing chromatin complexes.

Mechanism of H1

Early studies of the role of H1 clearly implicated the carboxyl Terminal Domain of H1 as necessary for the association of chromatin into higher order structures.[20] This effect has been attributed to the high levels of basic amino acids present in the CTD which would be capable of neutralizing the negative charge of DNA. However, Jeffery Hansen has shown by partial deletion of the CTD that the function of H1 is not due solely to charge neutralization across the entire CTD.[21] He has further shown that while the initial binding of H1 to chromatin has some electrostatic component, specific sub-domains of the CTD are responsible for stabilization and self-association of H1. These sub-domains can be topologically rearranged within the CTD without affect on the overall function of H1.[21] This finding is interesting in light of the usual requirement of proteins to be precisely ordered to function properly within the cell.

Through studies with *Tetrahymena*, Gorovsky has elucidated another portion of the H1 story. He has shown that phosphorylation of the linker histone regulates gene expression. However, unlike the specific post-translational modifications of the core histones which give rise to explicit alterations in chromatin structure and gene expression, phosphorylation of H1 seems to function through a less specific mechanism.[16] Tetrahymena H1 contains a 20 amino acid stretch in the amino terminal region which has five phosphorylation sites. Gorovsky mutated these sites so as to mimic either a constitutively phosphorylated or unphosphorylated state of H1. The constitutively phosphorylated form produced similar results as seen in the knockout experiments of H1 with an increase in ngoA levels and decrease in CyP1 levels. The unphosphorylated form showed increased expression of Cyp1.[16] Gorovsky further found that the alteration of transcription levels mediated by H1 was due to the creation of a charge patch by phosphorylation and not simply due to an alteration of the hydrophobicity or recognition of the phosphate.[22] These studies are relevant to the function of H1 because as the cell undergoes a transition through various physiological conditions, the phosphorylation

of H1 may increase or decrease, thus allowing a mechanism for control.[23] In the unphosphorylated state, H1 is able to strongly bind linker DNA and thus compete with other DNA binding proteins for access to regulatory sequences, thus, in some cases, activating transcription. Conversely, in the phosphorylated state, a negative charge patch is introduced which weakens H1 interaction with linker DNA and either allows regulatory proteins to bind the DNA or even allow the nucleosome to shift and thus expose a regulatory sequence which was previously inaccessible to binding proteins.[22] Additionally, Gorovsky found the location of the negatively charged region could be altered with no impact on gene transcription.

The results of Gorovsky and Hansen regarding the mobility of functional domains within the H1 protein make sense in light of the large divergence of sequence across species. Whereas core histone sequences are well conserved and modifications are specific, it seems that in H1, the precise sequence is unimportant in respect to the amino acid composition of specific domains. While the findings discussed above provide a great deal of insight into the function and mechanism of H1 inside the cell, a vast number of questions are unanswered. For example, one would like confirmation of the charge patch mechanism in an organism exhibiting a tripartite H1 structure. The topology of H1 association with the nucleosome to confer stability to chromatin is still poorly understood. Additionally, multiple other roles for H1 are likely in light of the H1b studies in mice, ATP-dependent chromatin remodeling complex interactions, and homologous repair inhibition. Overall, the study of H1 has implications not only in chromatin structure and gene expression, but also in cancer, aging, and development.

Physarum polycephalum as a Model Organism for the Study of Chromatin

The organism *Physarum polycephalum* has several specific biological properties which make it an ideal organism for the study of chromatin structure and function. During the macroplasmodial stage of *Physarum*, the several million nuclei contained within a single cell are perfectly synchronous throughout the cell cycle.[24] This property not only allows the elucidation of chromatin events at specific cell cycle stages, but also vastly enhances the ability to detect these changes because one is able to observe a huge population of nuclei. Additionally, exogenous proteins applied topically to the cell are absorbed and transported to the appropriate cellular compartment.[25,26] The utility of this property in chromatin study has been observed through the incorporation of introduced histones into the chromatin of the living cell.[27] This ability circumvents traditional requirements of genetic manipulation and microinjection; furthermore, it allows the introduction of biochemically relevant levels of modified proteins into the cell.

Previous Work on H1 in *Physarum*

Before much of the advanced laboratory methods for studying chromatin structure and function were available, *Physarum* provided an invaluable resource for researchers involved in this field. Because of its natural synchrony, and the large quantities of histones available from one cell, it was possible to perform studies on *Physarum* which at the time would have been impossible in other model organisms.

As early as 1973, Bradbury proposed that phosphorylation of H1 is temporally correlated with chromosome condensation and therefore with mitosis and cell division.[28] This result was obtained through measurement of the incorporation of radioactive phosphate at specific cell cycle stages. Bradbury saw a sharp increase in radioactively labeled H1 two hours prior to mitosis. Furthermore, the level of H1 phosphorylation was observed to drop off 20 minutes before mitosis was observed through phase contrast microscopy.[28]

Bradbury further extended these findings to hypothesize that phosphorylation of H1 was the initiation step of mitosis. He shows the phosphorylation of H1 is directed by an increase in the levels of phosphorylating activity, which corresponds with an increase in phosphorylated H1. This result shows that H1 phosphorylation is a consequence of a regulated enzyme activity and not merely due to an increase in substrate concentration.[29] If this phosphorylation activity did cause chromosome condensation, H1 could be implicated in the control of cell division which would in turn have broad implications in cancer biology.

To confirm the hypothesis that phosphorylation of H1 controls mitosis, in 1976 Bradbury used a heterogeneous chromatin extract of Ehrlich ascites containing Growth Associated Histone Kinase (HKG). In vitro, the extract was shown to phosphorylate calf thymus H1.[30] When the extract was added to *Physarum*, mitosis was advanced by twenty minutes. This result could indicate that it was indeed the phosphorylation of H1 which promoted mitosis. However, any experiment using an extract rather than purified protein must be considered carefully as the result of the experiment may be due to several factors present in the extract. Furthermore, the level of extract added was many times higher than the activity of HKG normally present in the cell. Considering this high level of enzyme and the relatively short time of advancement, these experiments provide little evidence that H1 phosphorylation is causatively connected to chromatin condensation.

Fischer found further confirmation that H1 phosphorylation occurs prior to mitosis in 1980. However, no dephosphorylation was seen prior to the onset of mitosis.[31] Futhermore, through studies with affinity chromatography on a DNA-cellulose column, Fischer showed that phosphorylated H1 is less strongly bound to DNA than the unphosphorylated form.[31] This finding seems to suggest that phosphorylated H1 is not the determining factor for the onset of mitosis. The release of H1 by phosphorylation could allow other factors to bind the DNA competitively, and thus enable modification of chromatin structure.

Early results involving H1 phosphorylation and the correlation to the onset of mitosis do not provide satisfactory evidence of a mechanism of these events, nor do they provide even a direct causal relationship. The basic nature of H1 is the major property which allows binding to DNA; thus, it makes sense that phosphorylation, and thus the introduction of negative charges to the H1 protein, would loosen interaction with DNA. How this would introduce a condensation event is unclear unless the release of H1 provides the opportunity for other condensing proteins to bind. As H1 has been shown to stabilize the intrinsic ability of the core histones to condense into higher order chromatin structures, and as no other stabilizing protein has been found to bind to chromatin, it

seems unlikely that it is, as early studies suggest, the release of H1 by phosphorylation that causes the condensation of DNA. Despite the conflicting results, early studies provide a solid basis of the utility of *Physarum* in the study of H1.

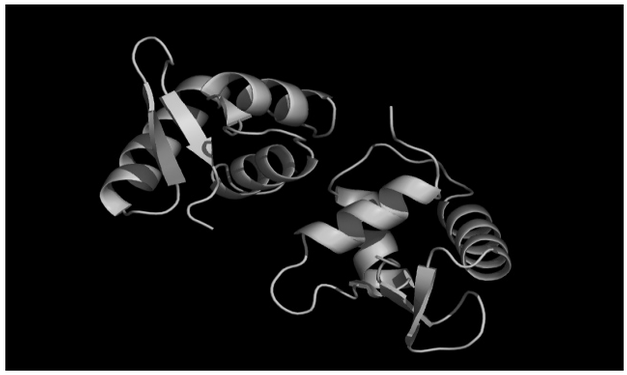
New Approaches to the Study of H1 in *Physarum*

The easy availability and more sophisticated laboratory techniques available for studies of yeast and other lower organisms shifted attention away from *Physarum* in the late 1980's. However, the properties of *Physarum* remain an untapped resource for the elucidation of chromatin structure and function at specific cell cycle stages. One of the *Physarum* characteristics which is useful in biochemical analysis is the ability to incorporate exogenous proteins placed on the surface of the macroplasmodium. Hayes and Thiriet studied the ability of *Physarum* to incorporate three different somatic linker histones into chromatin. Each linker histone was localized into the nucleus and incorporated into the chromatin.[27] It was also shown through salt release experiments that exogenous linker histone is less tightly bound during S phase than during G2 phase of the cell cycle. Additionally, when linker histone was incorporated during G2 phase a decrease in DNA transcription level was observed.[32] This moderation of transcription was dependent on the type of linker histone, with H1 decreasing transcription by 60% while H1° and H5 showed 30% reduction.[32] These findings are interesting in light of the lack of a G1 phase in *Physarum*. Thus, the cell must prepare for the events of DNA replication which occur during S phase before mitosis. This consideration may explain the delay of mitosis and requirement for high levels of H1 phosphorylation observed previously in *Physarum*.

Studies in *Physarum* have been retarded relative to those in yeast or *Tetrahymena* due to the absence of highly characterized genetic resources. However, recently a high titer cDNA library has been created by our lab; this library contains the DNA sequences of protein coding regions of the *Physarum* genome. Furthermore, *Physarum* has recently been selected for genome sequencing. The combination of the genome sequence and the cDNA library should propel research in *Physarum* as these resources will allow the power of *Physarum* as a model organism to be further realized.

Specifically, our lab has also screened the library for the complete coding sequence of the *Physarum* H1 protein. This sequence will allow further studies of the mechanism of H1 to be addressed in vivo. As previously described, *Physarum* incorporates exogenous proteins into chromatin. With the complete coding sequence of H1, it will be possible to mutate this sequence at specific locations and determine the effect of these mutations on chromatin structure and function at specific cell cycles. *Physarum* is well suited to these studies because unlike mammalian systems, only one subtype of H1 is present. This allows a more precise analysis of the effect of H1 on chromatin as there are no compensatory effects by other H1 subtypes.

In using these methods, it should be possible to examine the effect of phosphorylation of H1 because specific residues can be modified to assume a phosphorylated or unphosphorylated state and thus determine which phosphorylations are important to transcriptional regulation. This should allow further testing of the proposed charge patch mechanism proposed in *Tetrahymena*. Additionally, further investigation of the role of H1 in chromatin condensation, the regulatory events that promote H1 phosphorylation, and the global and local results of this phosphorylation will be possible through the study of H1 in *Physarum*.



Cartoon structure of Histone H5

Conclusions

Although the scientific community has made great advances in determining the function and mechanism of H1 in chromatin structure and function, much remains unexplored. As in the case of many biological systems, the field of chromatin studies was once seen as a rather unimportant aspect of gene function and regulation. However, as current studies advance, it is obvious that the role of chromatin is far more vital than simply DNA condensation. Studies of H1 provide insight into the role of DNA and chromatin binding factors, which provide the precisely regulated control of transcription. Certainly, these findings have broad reaching effects for the control of human pathology and aging. Through the development of H1 studies in *Physarum*, we are one step closer to unfolding the complex web of H1 interactions in the cell.

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The Need for Transformation:

A Challenge for Trauma Surgery

Joshua Brown, 2004

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With over 157,000 injury-related fatalities and nearly 11.5 million years of potential life lost in 2001,[1] traumatic injury remains a formidable challenge for medicine today. Fortunately, the field of trauma care has been rapidly advancing. Notable is the acceptance of nonoperative management of the trauma patient, which has become more prominent in the last decade.[2-4] This changing standard of care has greatly impacted the surgical resident's experience in the field of trauma.[5,6] The declining operative management combined with the newly enacted limitation of resident work hours and reimbursement deficits has raised concerns over the competence and recruitment of future trauma surgeons. To continue providing optimal treatment to the injured patient in the face of the rapidly evolving field of trauma care, a transformation is necessary in the training of future trauma surgeons. Several solutions have been proposed, and a consideration of the merits and limitations of each may serve as a catalyst for research and action regarding this need for transformation.

A Changing Field

Although organized civilian trauma systems have been developing throughout the second half of the twentieth century, only in the last twenty-five years have the benefits of organized trauma care been adequately recognized and implemented.[7] Trauma surgery today is vastly different from the field at its inception. The recent changes in diagnostic modalities, reimbursement, and resident work hours are powerful contributors to the current state of trauma surgery.

As a result of innovations in diagnostic modalities, an increasing number of trauma patients with mild to moderate solid organ injury are being managed nonoperatively. The widespread availability of Computed Tomography (CT) scanning and ultrasound have replaced diagnostic peritoneal lavage (DPL) as the primary diagnostic tools in trauma.[5,8] Previously, if DPL was positive the patient underwent an exploratory laparotomy. Many of these resulted in negative laparotomy findings and presented a host of complications in the guise of infection, wound management, and abnormal immune response with little benefit gained. Now, however, the non-invasive modalities of CT and ultrasound can detect free fluid, active hemorrhage, and the severity of injuries sustained, allowing surgeons to discriminate candidates for nonoperative management.[4] These patients are closely monitored and have a low risk of excessive transfusions, missed intestinal injuries, or delayed bleeding.[4] Nonoperative management has since become the standard of care for stable patients with splenic, liver and renal injuries in the setting of blunt trauma.[4,6,8] To provide perspective, the National Trauma Data Bank 2003 Annual Report shows that more than 80% of trauma patients from 1997 to 2002 presented with a blunt mechanism of injury. Furthermore, recent studies have suggested that nonoperative management is useful in specific subpopulations of penetrating trauma patients.[2,3] Although not in widespread application, these studies, along with the field of interventional radiology, broaden the potential for nonoperative management of trauma patients with solid organ vascular injuries by utilizing pharmaceuticals to promote local and selective blood clotting and prosthetic intravessel devices that stabilize damaged vessel walls, known respectively as angioembolization and stenting.[4,8] High frequency ultrasound has also shown promise as a method of vascular hemostasis in traumatic injuries.[9] The increasing nonoperative management of trauma patients represents a major shift in the care for the injured and has emerged as a dominant and growing treatment strategy for trauma surgeons.

Trauma surgeons have experienced a decline in fiscal reimbursement in recent decades, due to the increasing involvement of subspecialties, particularly orthopedic surgery, neurosurgery, and radiology in the care of trauma patients. Despite the greater investment by trauma surgeons in terms of clinical time, a disparity exists between the reimbursement of subspecialty physicians and trauma surgeons.[10] The discrepancy arises due to the greater time spent coordinating and delivering pre- and post- operative care, which is poorly or not at all reimbursed. In contrast, the involvement of subspecialties is almost exclusively spent performing procedures that are readily billable.[10] These findings come from a sample consisting almost entirely ofblunt trauma patients. In light of the commonly higher proportion of blunt trauma cases and the increasing nonoperative management of these patients, the inequity of reimbursement will only increase in the absence of rectification.

The limitation of resident work hours by the Accreditation

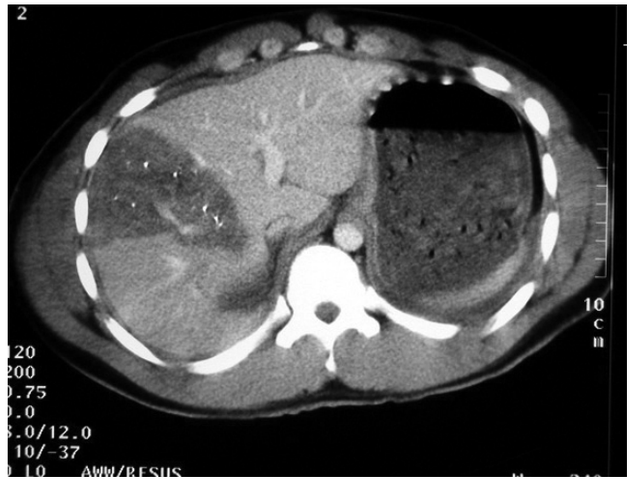
Council for Graduate Medical Education is a controversial change in the field of trauma surgery. When first suggested, the American College of Surgeons denounced the limitation of resident work hours in the field of surgery.[11] The movement began in the mid 1980s with the highly publicized death of Libby Zion, when the Bell Commission cited sleep deprivation and excessive work hours as a significant source of physician error—a conception that has since been severely challenged by the surgical community.[12,13] Despite the debate, the limitation of resident work hours represents one of the most sweeping changes not only for trauma surgery, but also for medical education in general.

Implications

The implications of these changes have raised serious concerns in the trauma community. The emergence of nonoperative management and work hour limitations has led to decreased operative experience for surgeons in training. Citing this decline in experience, some have called into question the competency of future trauma surgeons. Others are concerned that as a result of low reimbursement rates the recruitment of future trauma surgeons will suffer.

Due to the diminishing exposure of operative trauma, concern regarding the technical competency and confidence of future trauma surgeons has spread.[8,14,15] Engelhardt and colleagues found that general surgeons experienced a sixty percent decrease in thoracotomies and laparotomies over the last decade at their institution.[16] This decrease represents not only a large decline in overall procedures, but more importantly, a lost opportunity to educate residents on techniques and pitfalls during these procedures. The Residency Review Committee for Surgery has recognized this shift in trauma management by decreasing the number of index operative trauma cases from sixteen to ten, and by requiring an additional minimum of twenty cases managed nonoperatively, under a new category of Major Organ Trauma No Operation Required (MOTNOR).[17] Furthermore, a survey of residents by Barden et al. found that nearly half believed that the work hour limitations decreased their operative time.[12] Lewis suggests that trauma operative experience will be negatively impacted because of its non-elective nature, and argues that the institution of more didactic instruction is inadequate in addressing this problem.[13] As surgical trauma patients become rarer, valid concern over the competency and confidence of future trauma surgeons, who are only required to manage ten trauma patients with major operative injuries during their residency, is raised. Will the trauma surgeons of the future, who have a background of primarily nonoperative management and less overall exposure to operative cases, be able to provide optimal care to the catastrophically injured patient requiring immediate and substantial surgical intervention?

While the technical capability of trauma surgeons cannot be underestimated, it may be irrelevant if current trends continue. As a result of the changing field of trauma surgery, there is a significant lack of interest in trauma as a career. The entire field of surgery has suffered a lack of new recruits. With the issues of lifestyle and the push for more generalists, the number of unfilled general surgical programs has risen from five in 1997 to twenty four in 1999.[18] The field of trauma has borne the brunt of this problem, evidenced by only 58% of



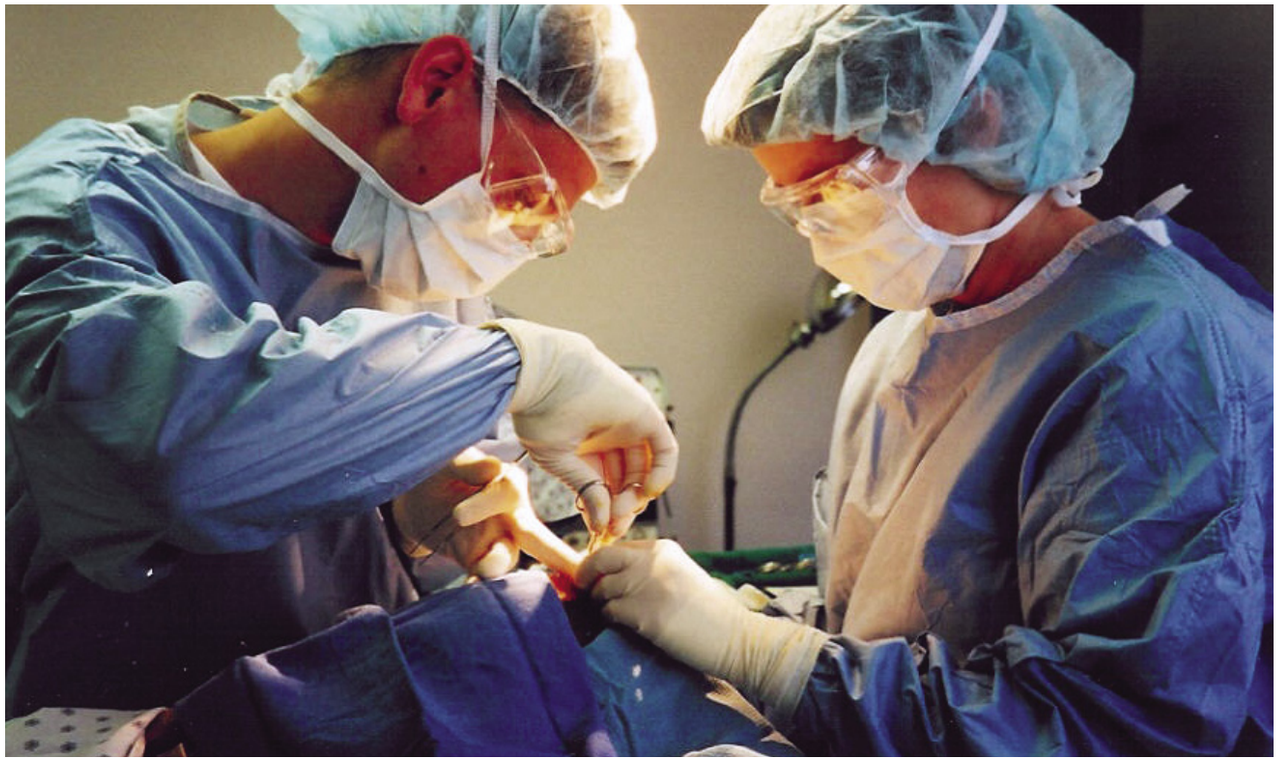
Liver injury secondary to a stab wound to the lower chest on CT Scan.

trauma fellowship positions filled in 2002, a decline from previous years.[14,19] The lack of operative experience has been a lament for many authors. In 2003 a multi-institutional study noted that the laparotomy rate for trauma admissions is near 5%, and may be less in centers that receive primarily blunt trauma.[20] Low operative experience combined with a high inpatient admission rate is cited as a major deterrent to a career in trauma.[10,21] Similarly, the low reimbursement rate when compared to the high workload and numerous on-call hours makes trauma surgery an unattractive career choice in its current state.[22] This myriad of factors threatens the field of trauma surgery with a troubling shortage of talented surgeons, thus jeopardizing optimal care to the injured.

Proposed Solutions

To address the legitimate concerns regarding the future of trauma surgery, several solutions have been proposed to maintain operative competency and increase the appeal of a career in trauma to young surgeons. These propositions all aim to transform the field of trauma surgery and have taken the form of new models and new training techniques, each with distinct merits and limitations. Highlighting these advantages and disadvantages may serve to catalyze further investigation into the need for a transformation in trauma surgery. One of the most popular models that have been suggested is that of the Trauma/Emergency Surgeon. Under this model, which reflects the current functioning of trauma surgeons in some institutions, the role of the trauma surgeon would be expanded to include care of all general surgical emergencies.[14] The underlying principle is to increase the operative procedures performed by trauma surgeons, thereby maintaining their operative skills. Further, it is hoped that this increase in operative procedures will make the field more attractive to residents and result in better compensation rates.

Scherer et al. found that this model, when applied in a large urban trauma center, more than doubled the operations performed by trauma surgeons.[21] However, to achieve this growth in operative experience, trauma surgeons had to admit twice as many patients as general surgeons with an elective practice, had to perform nearly a fifth of their operations at night, and had a significantly poorer payer mix than elective surgeons. This raises concern that while increasing the



A team approach is essential in complex operative trauma.

operative experience in favor of increasing surgeon competency, the increased workload, high nocturnal activity, and poor reimbursement remain as deterrents to young surgeons. The authors advocated pay for on-call time to offset the poor payer mix. They further argued that the increase in operative experience is sufficient to expand the number of trauma surgeons on staff, thus reducing on-call time for individual surgeons. This model is best served by urban academic centers where there is a steady supply of trauma and general surgical emergencies.[21] In a rural hospital the volume of trauma and surgical emergencies may not be sufficient to warrant the trauma/emergency surgeon. This model successfully increases the operative experience and competency of trauma surgeons; however, if the high workload and poor reimbursement are not addressed, it does little to increase the attractiveness of the field.

A second model proposed is that of the trauma specialist. This model purports to focus interested residents narrowly into trauma, and includes surgical critical training, creating super-specialists in the care of critically injured patients.[14] It is designed to reduce the number of trauma positions by further concentrating trauma care in regional trauma centers, creating a smaller group of elite trauma surgeons. In theory limiting trauma positions and further concentrating trauma admissions would reduce trainee competition for operative experience in trauma. The further hope is that the identification of trauma and critical care as a distinct practice would increase the attractiveness of the field as with the subspecialties of vascular and colorectal surgery. This is seen as an approach for attracting talented and competitive surgeons to a limited number of positions.

Support for this model comes from the acknowledgment of over-designation of trauma centers and the success of the R. Adams Cowley Shock Trauma Center in Baltimore, Maryland.[23] The American College of Surgeons (ACS) verifies trauma centers based on in-hospital resources rather than regional needs, resulting in lower trauma admission rates for centers in areas of over-designation. In contrast, the Shock Trauma Center is a truly regionalized dedicated trauma center. Receiving more than 5000 trauma admissions annually from Maryland and surrounding states, surgeons are exposed exclusively to a high volume of trauma patients. This results in greater operative trauma experience and fosters committed leaders and researchers in the field of trauma surgery. Furthermore, although controversial, the appropriate regionalization of trauma systems has been implicated in improving patient outcomes.[7,23] Despite these arguments, there are several drawbacks to this model. Reducing the number of positions in trauma will not necessarily translate into competition between talented surgeons, as evidenced by the high percentage of unfilled fellowship positions discussed above. Differing regional needs across the country may render this regionalization of trauma care inappropriate. The success of Shock Trauma suggests that such regionalization is suitable in densely populated areas with a well developed emergency medical system to support it. To achieve such regionalization would require significant logistical, political, and fiscal commitments. Moreover, the decrease in trauma positions and concentration of critically injured patients in regional centers would necessitate greater time commitment on the part of the training surgeon, thus becoming a potential barrier because of lifestyle concerns and resident work hour limitations. Finally, the trauma population tends to be a poor payer mix, as discussed above, and this model makes no attempt to rectify the reimbursement problems for trauma surgeons. While this model contains some attractive aspects for the field of trauma

surgery, it more likely represents a long term goal with limited areas of application that can adequately support it.

A third model proposed is the so-called European Model. In this model, trauma surgeons would have the credentials in performing orthopedic and neurosurgical procedures common in trauma patients.[14] Trauma surgeons would spend an additional year of training in each subfield learning procedures such as external fixation, intracranial monitor placement, and burr holes.[10,14] In doing this, trauma surgeons will be performing more operations, which would result in an increase in attractiveness and reimbursement rates for the field. Further benefits of this model include the continuity of care associated with a single surgeon operating on the patient and the ability to loosen ACS verified trauma center availability requirements on orthopedic and neurosurgeons. This appeals to some of these surgeons who have expressed dissatisfaction in caring for trauma patients.[10]

This model dominates trauma care in Europe, with noted success.[14] However, there are several challenges that face the implementation of this model in the United States. This model adds operative skills to the inventory of the trauma surgeon instead of preserving skills in the staple of the trauma surgeon: abdominal surgery. This leaves unaddressed the concerns over trauma surgeon competency. Another issue is validation and accreditation for this extra training. With both orthopedics and neurosurgery under the direction of individual boards, care must be taken to respect the autonomy of the distinct specialties. Additionally, it is questionable that trauma surgeons could reach an acceptable level of proficiency in these fields with only an additional year of training in each, when compared to the residency length for orthopedic and neurosurgical residents. This presents the possibility for increased legal action in an arena where competency would potentially be controversial. Further, the additional two years of post-graduate training would be a deterrent to some. It is also conceivable that implementation of such a model would meet some resistance to established trauma surgeons who have no interest in expanding their practice. Although this model raises overall operative experience and serves the field of trauma surgery well in Europe, the specialties of neurosurgery, orthopedics, and trauma surgery have developed in the United States such that implementation of this model would be drastic and meet considerable resistance, requiring significant political and professional support.

In addition to the three models proposed, there has been support for new training techniques to boost surgeon operative competency and confidence. The value of interactive training in the management of trauma patients has been recognized since the advent of the Advanced Trauma Life Support course. Recent advancements in simulator technology have made possible the application of sophisticated computerized simulation modules in the field of trauma surgery. This often includes a lecture review of management principles followed by computerized human patient simulation scenarios, which several authors have supported as valuable training tools.[25-28] In addition to identifying training weaknesses, these simulator courses have been shown to increase the confidence and knowledge of residents. While thus far restricted to initial patient management and critical care scenarios, it is possible to expand these simulators for use in operative management scenarios for trauma, increasing operative knowledge and confidence in future trauma surgeons. The major limitation of this method is the lack of actual technical experience in computerized simulations.

Another proposed training technique is a trauma operative management course. This also includes didactic portions and is then followed by a live porcine operative experience. Similar studies evaluating this type of course found that an operative management course increased the knowledge, skills, and confidence of trainees and experts during operative management of penetrating swine injuries.[15,24] This method again increases knowledge and confidence of surgeons when managing operative trauma, as well as addresses the lack of technical experience cited in the simulation training. The swine confronts the participants with the best approximation of the human scale and anatomy, and most report that the laboratory experience is sufficiently analogous to operative management of human trauma.[15] This suggests that the best training method may be a combination of simulation and laboratory experiences. While addressing the concerns of trauma surgeon competency, these are not transforming models for trauma surgery and do not address problems in reimbursement. It is also unlikely that implementation of such training techniques will greatly increase the appeal of trauma surgery as a career. The merits of these techniques warrant their inclusion as a component in the overall transformation necessary in trauma surgery, rather than a stand-alone solution.

Conclusion

The goal of trauma care is to provide optimal care to the injured patient. To achieve this, the field of trauma surgery must attract talented surgeons and equip them to treat any pattern of injury they may encounter in their practice. Without the changes in management, reimbursement, and work hours, trauma surgery in its current state is falling short of these requirements.

Although a recent study found no significant overall mortality difference between high and low volume trauma surgeons, the authors noted that the effect of experience on mortality may lie in the management of specific injuries, and indeed the greatest difference in mortality was seen in the subgroup of patients presenting in shock with abdominal gunshot wounds.[29] This trend suggests that the true effect of experience on mortality may lie with patients requiring operative interventions. In addition to the likely effect on mortality, there is a potential for even greater effects of operative experience on outcomes, such as length of stay and intensive care unit days that may be valuable in characterizing the significance of trauma surgeon competency.

The literature has suggested several solutions that address the problems of decreasing operative experience, interest, and work hours in diverse ways, creating unique advantages and disadvantages for each. This renders it erroneous to assume that any one solution can enact the transformation that the field of trauma surgery requires. Instead, it is the responsibility of the trauma community to combine, tailor, and refine the proposed solutions in an effort to preserve the delivery of optimal care to the trauma patient.

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Coral Reef Bleaching:

Linking Ocean Optics with Coral Health

Kaccie Li, 2005

Advised by James Zavislan, Ph.D.

Department of Optics

In this study, we took a new approach in correlating the optical properties of various seawaters to coral reef health. After analyzing the data obtained in various optical oceanography publications, we found that there is a significant correlation between the optical properties of water to the apparent health of the coral reefs. Coral reefs around the world have an important role in keeping the ocean chemically and biologically stable. A large enough disturbance to a reef's dynamic system can result in incorrigible consequences. Issues such as global warming, over-fishing, pollution, and the ozone hole are several popular items subjectively mentioned to be associated with coral depletion. Studies concerning both coral health and optics are rare. This is especially true when assessing how the sun's spectrum changes when it propagates through seawater. Since corals are light dependent organisms, we believe it is essential to bridge the gap between physical oceanography and marine biology when it comes to maintaining a healthy reef.

Bleaching is the process of corals not being able to sustain the crucial symbiotic photosynthetic algae within their tissues. The potential result of continuous bleaching has been labeled as a worldwide threat to the health of our fragile coral reefs.[1-3] Examining the measured inherent optical properties of seawater, one can see the variations in the underwater electromagnetic spectrum and approximate a value for the amount photosynthetic available radiation (PAR). Past and more recent data on ocean radiative transport theory was used to observe how the optical conditions corals experience have changed with time. Our results have shown that geographical locations known for bleaching are consistently different than those areas with a stable coral population.

Background

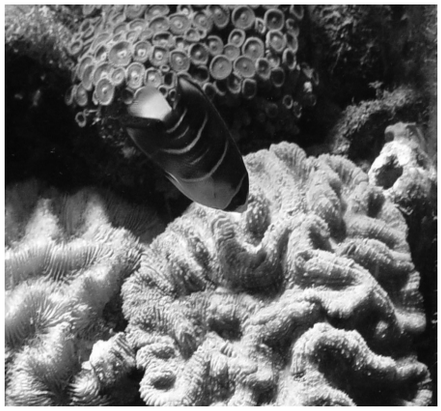
The survival of corals strongly depends on the concentration and health of their symbiotic intercellular chlorophyll-containing algae: zooxanthellae. These tiny algae are either captured directly by the coral polyps, or bred through a starter culture within the parent coral. Zooxanthellae are nursed safely inside vacuoles within the host coral's tissues providing the coral with vital energy directly from photosynthesis.[1,2] The observation that zooxanthellae respiration and oxygen production is dependent on light intensity and spectral distribution was made as early as 1967. For this reason, we focused our explanations primarily on how corals tend to respond to light and other radiations.

Literature in coral growth has been mostly biological with the exception of a several occurrences when laboratory experiment involving effects of light conditions were integrated in. As a whole, studies involving both corals and optics are rare.[3] In contrast, factors including temperature, seawater composition, biomass, and the chemical dynamics of dissolved organic matter (yellow substance) are well surveyed for seawaters. Much literature on coral growth exists within the subject of marine biology, and an incredible amount of research has been conducted in the subject of ocean optics.[2,4-6] Correlating ocean optics research with living corals has still resulted in very subjective and hypothesized explanations for coral depletion. In this study, we used the data available concerning the inherent optical properties of seawater to determine if there is a direct correlation between that and coral health.

Available experimental data representing coral growth under artificial lighting conditions mainly involve in vitro studies of several different kinds of corals under various filtered spectrums and have provided only a qualitative idea on a coral's optical needs.[2] Only fluorescent lamps and low quality filter glasses have been used, signifying that illumination consistency was weak with respect to wavelength. Our concerns with results from this type of study come from the fact that no attention was brought to the output of the light sources used. Any fluorescent light source will contain sharp peaks in its spectrum, so results based on it cannot be a representative of sunlight. Experiments of this nature may produce consistent results pertaining to the responses of corals to certain types of illumination, but they do not provide much insight in what is causing coral bleaching in a natural setting.

Inherent Optical Properties of Various Seawaters

The amount of photosynthesis available radiation (PAR) and harmful radiation are responsible for a coral's health. PAR consists of wavelengths between approximately 0.4 to 0.7 microns. Because no precise data on the action spectrum of different corals (more precisely their symbiotic zooxanthellae) are known, we cannot yet quantitatively correlate dynamics in a coral varietie's response to different wavelengths within



The exposure of the white exoskeleton of the coral indicates insufficient algae concentration known as bleaching.

the PAR range. In "clear" seawater, PAR reduces to 1 percent of its incident value within 100 m. This metric is called the euphotic depth. Spectral distribution plots show that due to slight turbidity changes in different waters, the euphotic depth can extend from over 200 m to less than 40 m. This depth range is labeled as the euphotic zone.[6] Because of coral's PAR requirement, we may conclude growth promotion is the greatest in clearer waters (greater euphotic zone). Smith and Baker conducted research on the inherent optical properties of natural waters. Their data on the optical absorption and back scattering of the clearest seawaters is key in this study in that correlations can be made from seawaters from various regions to that of clear natural water.[5]

The sun's spectrum outside of the earth's atmosphere is near that of a blackbody at 5900 Kelvin. Optical transmission is wavelength dependent and varies with the continuously changing properties of the atmosphere. In-depth evaluation of atmospheric optics is beyond the limits of this paper. However, S. L. Valley's Handbook of Geophysics and Space Environments give estimated but detailed numerical values for the solar irradiance at sea level (at STP, 2.0 PR. cm water vapor, and 0.28 atm/cm ozone) for several zenith angles. This set of data also shows that the shape of this distribution remains nearly identical even with significant changes in the zenith angle. However, total irradiance about the visible spectrum will decrease to 50% of the maximum spectral irradiance when the zenith angle reaches around 70 degrees.[7] Radiation directly from the sun is first altered by the atmosphere, and at sea level, much of the harmful wavelengths have already been removed. At sea level, only about 4 percent of the incident light is reflected at the surface. We can then directly use the sea surface irradiance data and apply the effects of absorption and scattering as solar irradiance propagates to the reef bed.

The depths of coral habitats vary amongst species, but their similar PAR needs keeps this range very limited. Around where it is considered to be the clearest open ocean waters, the compensation depth of most corals (the depth at which coral respiration equals the product of photosynthesis) is safely within 40 m. Most corals are not expected to grow at depths beyond 15 meters.[3] In fact, most corals collected for artificial reef systems are located within the first 10 m.

Displaying the irradiance spectrum and calculating the available PAR with respect to seawater depth is a very simple process. Absorption and scattering coefficients are both wavelength dependent, and Smith and Baker tabulated them both for the clearest natural seawaters. We refer to Smith and Baker's data as our base spectrum model due to the empirical result that corals thrive in clear water.[3] Downward propagating light decreases exponentially in magnitude. The equation for spectral irradiance decay, $E_\lambda(\lambda) = E_{0\lambda}(\lambda)e^{-k(\lambda)z}$, is known as Beer's law. The diffuse attenuation coefficient k is simply the sum of each pair of absorption and scattering coefficients, and the wavelength dependent surface spectral irradiance value $E_{0\lambda}$ was tabulated by S. L. Valley. This simple relation allows us to display the optical spectrum and its attenuation behavior with respect to the depth z. Finally, integrating the spectral irradiance from 0.4 to 0.7 microns is the PAR.

When discussing the optical properties of natural ocean waters, the variables we are concerned about are biomass, contaminates, composition, and sedimentation. N. G. Jerlov developed arguably the most complete classification of ocean waters based on their optical properties. Jerlov published his world plot of water types with tabulated optical transmission information in 1976. More recent world plots done by satellite imaging for the most part agree with Jerlov's original plot, so different plots can be used simultaneously to analyze potential significant changes in the oceans' physical properties. Due to the dynamic nature of natural occurrences and human impacts, water type classification will continuously vary with time,[6] which suggests that this classification is highly subjective for short time intervals. This was not the case for the available plots that are nearly 20 years apart. Correlation of Jerlov's original plot from 1968, when concern for the bleaching of coral reefs was nonexistent, with a more recent version published by Simonot and Le Truet in 1986 shows that seawater optical transmission have significantly decreased in many areas.[4,6]

Results and Conclusions

Jerlov water types (JWT) are represented by Roman numerals for open ocean waters and Arabic numerals for coastal waters. The clearest open ocean waters are labeled JWTI, while the most turbid open ocean regions are JWTIII. The clearest to most turbid coastal waters come in one level below JWTIII and are labeled from JWT1 through JWT9 with JWT1 being the clearest. Due to the fact that many large ocean regions have optical properties that fall between JWTI and JWTII, JWTI has two subsets labeled JWTIA and JWTIB. Irradiance transmission coefficients for JWT are tabulated in *N. G. Jerlov's Optical Oceanography.*[4] Like the diffuse attenuation coefficients, they are wavelength dependent, but identical to light behavior at an interface, they represent the percentage of light that transmits through the medium. Jerlov's separation for coastal waters and open ocean indicate only that in most cases, coastal water are more likely to be heavily affected by contaminants from landmasses. It is very possible that many open ocean regions today are classified as Jerlov's coastal water types.

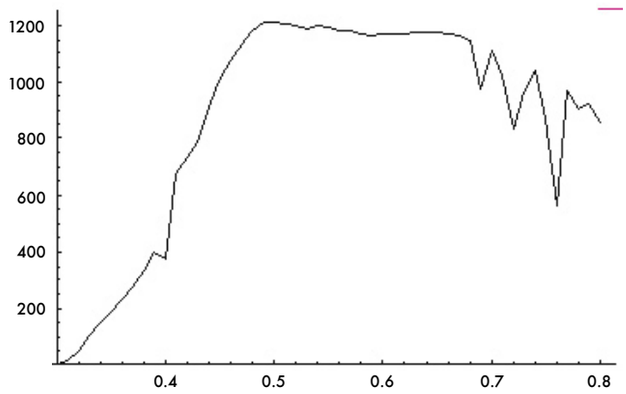
According to Jerlov's regional distribution of optical water types, the Great Barrier Reef and many other major coral reefs are located in IB waters.[4] One can see that the spectral distribution shape for IB seawater is very similar to that of pure water. IB seawater, by observation, absorbs more UV and PAR and less IR than pure water. However, it is clear that the differences between the two are nominal. In IB waters, the chlorophyll concentration is about 0.1 mg/m^3. This is an insignificant amount to consider optical scattering solely due

to the chlorophyll content in the water.[6] However, since the bleaching of coral reefs was not a recognized environmental problem until the 1980s (12+ years after Jerlov's publication), it is very possible that IB seawater type provided the ideal optical properties of seawater for healthy coral growth. So perhaps the concentration of phytoplankton, dissolved organic materials, other microorganism, etc. in IB water types had little effect on the PAR distribution at the coral beds, but they provided an adequate concentration of free-moving nutrients and zooxanthellae essential to the healthy coral. Also, increases in chlorophyll content (coming with an increase in dissolved organic materials) correspond to sharp decreases in euphotic, or survivable, depth.[6] This correlation suggests that the dynamics of natural seawater is indeed fragile, since many more variables exist to disturb the its optical properties.

Seawater turbidity data provided by Simonot and Le Truet from 1986 classifies most of the Great Barrier Reef region as JWTIII and II. Only significantly outside of mainland Australia does the seawater become IB. This is strongly opposed to Jerlov's earlier data claiming this region to be IB with only a small portion of the barrier reef classified as II.[4,6] The spectral irradiance distributions at JWTIB, II, and III at various depths show that the spectrum at their same relative depths are similar, but they contain relatively less PAR as turbidity increases. PAR value at the sea surface sits at about 336.1 W/m^2, and at 3 meters below the surface in type IB waters, PAR drops to approximately 65 percent of the surface value. Water types II and III would have only 59 and 50 percent of the surface PAR value when measured 3 meters below the sea surface. Experimental results off the reef system in the Florida Keys claim that the increase in seawater turbidity is responsible for the depletion of coral cover in that area.[3] Their PAR irradiance measurements, done by a LiCOR sensor, shows that during clear summer days, the irradiance at 3 meters is around 53 percent, which is nearly the value of type III waters! We believe this correlation is not a coincidence. If such optical conditions do not provide sufficient compensation depths, then it is highly probable that coral depletion in the Great Barrier Reef region is a direct consequence of unacceptable turbidity levels.

Recent research expeditions have found densely populated coral beds in depths beyond 30 meters. The surprising discovery is that deep-sea corals are thriving while a considerate amount of their shallow water counterparts are struggling to survive.[8] As mentioned earlier, previous criterions claim that corals are not expected to grow at depths beyond 15 meters.[3] In the clearest seawaters, the proposed compensation depth for corals is up to 40 meters, but surely it is vexing that these deep-sea corals are thriving at depths past 30 meters. If these coral do not require the same quantity of light to thrive, it must mean they have another source for energy, unless deep-sea coral only need the small amount of solar energy available to them. Recalling Beer's law for light attenuation, any exponential decay rate decreases as its argument depth increases. The values of diffuse attenuation coefficients of different water types are not exceedingly different according to our models. So at depths greater than 30 meters, it can be postulated that the amount of PAR is not significantly different as to when dealing with depths of several meters. This should be considered for further research in this topic.

In this study, results derived from several disparate data sources have provided strong evidence that a modest change in the optical properties of seawater affects the health of tropical coral reefs.[2,3,7] The JWT classification metric, diffuse attenuation coefficients, and field instruments such as the LiCOR give us a way of modeling the quality of light (most importantly PAR) available to corals in different locations and depths. Data from JWT classifications 15 years apart have justified that seawater turbidity has noticeably increased in the Great Barrier Reef region, as well as several other coral reef regions, as a highly probable cause of coral cover depletion. Data collected from the Florida Keys correlated well with other problematic coral regions when the calculated PAR are compared for further evidence on this claim. This correlation must mean that proper optical conditions need to be provided in order to sustain the life of our coral reefs.



Irradiance spectrum at sea level given the atmospheric conditions in the text.[7] (units: W×m^{-2}/μm vs. m)

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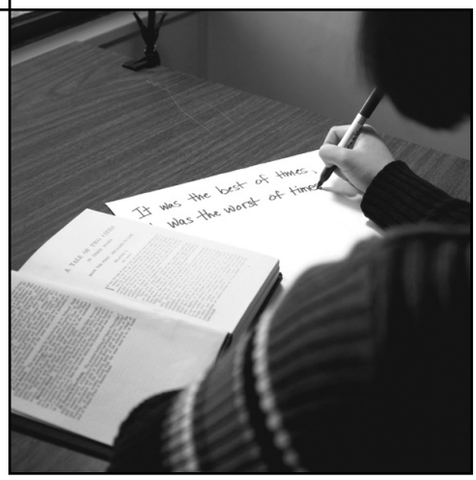
Kaccie Li will complete his B.A. in Mathematics and B.S. in Optics in May, 2005. He expanded upon a hobby involving marine aquariums to make this project possible. He plans to pursue an O.D./Ph.D. degree and further advance the vision correction field.



Being Mindful:

Facilitating Enhanced Personal Integrity and Interpersonal Honesty

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Josh Felver-Gant, 2005

Advised by Kirk Warren Brown, Ph.D.
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Honesty as a psychological construct is both poorly defined and insufficiently understood. Increasingly, however, honesty as a dynamic in interpersonal relationships has come under closer scrutiny and is recognized as an essential component of healthy and meaningful relationships of all forms.[1] Rogers conceptualizes honesty in terms of the state of congruence in which an individual does not deny himself the feelings being experienced and is willing to express them and be open to others.[2] "During an honest moment, the individual experiences what is available to his awareness."[1a] In an honest interaction, the experience will be "transparent," or available to each person's awareness. Honesty is therefore defined by a close matching of what is being experienced and what is being expressed by the individual.[3] Since honesty plays such a vital role in establishing and maintaining interpersonal relationships, it must be employed in order to foster self-growth and to develop healthy and meaningful relationships. This emphasis on individual experience and awareness of honesty in interpersonal communication has guided the present research in its study of the relationship between mindfulness and various forms of honesty.

Mindfulness is an element of consciousness that is characterized by a heightened state of awareness in which an individual is attentive to being in the present moment. This enhanced attention to and awareness of current experience or present reality has been shown to be associated with enhanced self-awareness, self-regulated behaviors, and positive emotional states.[4a]

Robert Sternberg has thoroughly examined the concept of mindfulness, and he considers three possible conceptions of it: 1) that mindfulness should be understood as a cognitive ability—people differ in their capacity to think in a mindful way, much as people differ in memory or intelligence; 2) that mindfulness is a personality trait, and is thus a stable disposition, much as would be extraversion or neuroticism; or 3) that mindfulness is a cognitive style that represents a preferred way of thinking.[5] He concludes that while mindfulness has characteristics of all three, it seems closest to being a cognitive style.[5] However, as Langer and Moldoveanu point out, "a style is not expected to change over time and through different circumstances, whereas the essence of mindfulness is change."[6a] They further assert that a particular cognitive style cannot be mindful by definition as sensitivity to the novel, and thus the unexpected, are key components of mindfulness. Sternberg agrees that construct validation is required to address the question of whether mindfulness is more static or unstable in nature.[5] Although many difficulties are associated with classifying the nature of mindfulness, the various measures of well-being associated with mindfulness have been analyzed and shown to be true of both dispositional and state mindfulness.[4b] Therefore, regardless of how one chooses to categorize the construct of mindfulness, it has been found to be associated with well-being.

It has been proposed that the self-endorsed behavior regulation found to be a consequence of mindfulness may be important in disengaging individuals from automatic thoughts, habits, and unhealthy behavior patterns.[7] Dishonesty in one's interpersonal relationships, for example, is a behavior pattern generally considered to be unhealthy. Overt measures of dishonesty generally examine admissions of dishonest acts, rationalizing dishonest behaviors, being lenient toward others who are dishonest, and believing that most people would engage in dishonest activity.[8] However, dishonesty may manifest itself in a variety of other ways associated with interpersonal relations, including deception both of oneself and of others.

Social desirability is a response style in which individuals tend to present themselves in such a way that makes them look better, or, alternatively, that avoids making them look bad.[9] There appears to be a dichotomy inherent in social desirability responding (SDR) between self-deceptive positivity, which is an honest but overly positive self-presentation, and impression management, which is self-presentation tailored to an audience. Impression management also appears to consist of both the enhancement of good traits as well as the denial of negative ones.[10a]

In the case of self-deception, it has been proposed that "in order to be self-deceived an individual must hold two contradictory beliefs; these beliefs are held simultaneously; one belief is not subject to awareness, and the unawareness of this belief is motivated."[10b] Although within psychology it is traditionally assumed that people are necessarily aware of their cognitions, the term self-deceptive implicitly prohibits such an assumption.[10c] The lack of awareness associated with self-deception would imply that less mindful individuals would tend to be more self-deceptive.

Awareness and, consequently, mindfulness may be applied to other forms of deception within an interpersonal context. More mindful individuals are generally perceived as being more genuine than less mindful individuals, and this audience reception has been shown to increase positive effect.[6a] Mindfulness in interpersonal communication should ultimately benefit both participants, whereas a lack of mindfulness in communicative practices "often results in misunderstanding and misperceptions among communicators."[6b] Thus, increasing mindful awareness in interpersonal communication can deepen and broaden social understanding.

Indeed, mindful individuals have been found to be more competent in conversational interaction. Studies by Waldron and colleagues showed that in conversing with a reluctant partner, competent interaction was characterized by using a "proactive and flexible approach that epitomizes mindfulness. Competent interactants were found to use more extensive and creative planning processes as they contemplated conversational moves."[11a] This included looking further into the conversational future, anticipating more developed alternative partner responses and being more responsive to the immediate context as they constructed conversational plans. This enhanced cognitive engagement tended to put conversational partners at ease and facilitated the achievement of information-seeking goals.[11b] This shows that greater mindfulness in planning and executing conversational goals may enhance genuineness in social interaction and lead to substantial benefits in practical interpersonal communication contexts.

The various positive outcomes associated with mindfulness as it relates to individual integrity, honesty, and the enhancement of effective and genuine communication with others leads the present study to predict that mindfulness will also be related to differential measures of honesty. This will be manifest in mindful individuals being less self-deceptive, less likely to respond in a socially desirable manner, and more likely to be honest in interpersonal communications. To test these hypotheses, various measures of self-deception, social desirability responding, and interpersonal honesty in communication will be implemented.

Participants in this study were selected from a general pool of psychology undergraduates at the University of Rochester on a volunteer basis. The ages of participants ranged from 18 to 23 (mean age = 19.17), with a total of 94 subjects, 58 of whom were female and 36 of whom were male. Upon completing the surveys, participants were compensated with chits redeemable for extra credit in their respective undergraduate psychology courses.

To operationalize the experiment's variables, five survey measures were used. The Mindful Attention Awareness Scale (MAAS) is a 15-item, 5-point likert scale (α = .8125) designed to measure one's level of mindfulness as demonstrated through one's attention and awareness to the present moment.[4a] To test for social desirability responding, the Marlowe-Crowne scale (1960), a 33-question true-false measure, was implemented (α = .6585). Two tests designed to assess honesty were also used. The Honesty in Interpersonal Relationships (HIP) scale designed by Snyder (1996) focuses on interpersonal honesty in different social contexts (α = .8113), and a 10-item subsection of the Values in Action Inventory of Strengths (VIA-IS) scale by was adapted for the current study (α = .8307) to measure honesty and personal integrity.[2]

Surveys were completed as part of a packet compiled and distributed by Dr. Kirk Warren Brown, which included several questionnaires of other Research Methods students, for their respective studies. Groups of participants were run in a room at the University of Rochester designated for the purpose through the Psychology department. Questionnaires were filled out via Scantron format with paper and pencil.

Descriptive statistics for the four measures employed are summarized in Table 1. Running a series of bivariate correlations with this data revealed, as predicted, a statistically significant correlation between mindfulness as determined by the Mindful Attention Awareness Scale and the two honesty measures, Honesty in Interpersonal Relationships, and the VIA-IS subsection Honesty/Integrity (HI) scale. However, there was no significant correlation between mindfulness and social desirability as measured by the Marlowe-Crowne scale. All correlations performed are shown in Table 2.

The HIP scale and the VIA-IS HI scales were both correlated with the MAAS. The strength of these relationships is demonstrated in a moderate correlation of .279 ($p < .01$) for mindfulness and interpersonal honesty, and a slightly smaller correlation of .217 ($p < .05$) for mindfulness and honesty/integrity. The similarity of these patterns of relationships and the fact that the values are very close statistically suggest that the scales may in fact be measuring the same trait, and that honesty and integrity may be linked. Further statistical analyses showed a strong significant correlation between interpersonal honesty and honesty/integrity, with a correlation value of .307 ($p < .01$). This correlation and the analogous results of the two scales provide an indication of the internal validity of the scales employed, as the scales appeared to measure the traits for which they were intended.

These results indicate that a heightened attention and awareness of the present moment as operationalized by the MAAS measure of mindfulness is strongly associated with higher levels of self-reported interpersonal honesty and

Table 1. Descriptive Statistics

	Minimum	Maximum	Mean	Std. Deviation
MCMEAN	.12	.70	.3861	.12991
HIPMEAN	2.90	4.50	3.7550	.31071
HIMEAN	1.00	5.00	3.9645	.58726
MASMEAN	2.07	5.53	3.8369	.65480

Valid N (listwise) 94

Table 2. Pearson Correlations

	HIMEAN	MCMEAN	MAASMEAN	HIPMEAN
HIMEAN	1	.172	.217*	.307**
MCMEAN	.172	1	.161	.178
MAASMEAN	.217*	.161	1	.279**
HIPMEAN	.307**	.178	.279**	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

attitudes regarding honesty and personal integrity. Social desirability as measured by the Marlowe-Crowne scale was not found to be correlated with mindfulness (r = .161). Further analyses determined that the Marlowe-Crowne scale was also not significantly correlated with either interpersonal honesty (r = .178) or honesty/integrity (r =.172) as measured in this study. This lack of correlation between the Marlowe-Crowne scale and these other measures of honesty suggest that social desirability is a separate phenomenon than honesty and integrity, which appear to be linked.

The present study assessed the prediction that mindfulness operationalized by the MAAS would be related to various measures of honesty. The results showed that, as predicted, mindfulness appears to be related to personal honesty and integrity as well as interpersonal honesty. However, inconsistent with the hypothesis, there was no clear relationship between mindfulness and social desirability responding as measured by the Marlowe-Crowne SDR scale. This suggests that mindful individuals are aware and attentive to the present moment and their emotional states, and that this heightened awareness facilitates honest and genuine interactions with others. The interpersonal experience is thus more likely to be "transparent" to the individuals involved and there will be a close matching between what a mindful individual experiences and what is expressed to the conversational partner, as Rogers has proposed.[3]

This finding is supported by studies done by Waldron and colleagues in which it was found that competent conversational individuals, or those who were best able to obtain sensitive information from reluctant partners in a manner that minimizes social discomfort, tended to be "more responsive to the immediate context as they constructed conversation plans," employing a "proactive and flexible approach, which epitomizes mindfulness."[11c] The authors concluded that "greater mindfulness prior to and during communication can accrue substantial benefits in a variety of important practical communication contexts," and one of these benefits appears to be an increase in interpersonal honesty.[11d]

The reason that mindfulness would be associated with honesty but not social desirability is not immediately apparent; intuitively, self-deception and impression management would seem to be inherently linked with forms of dishonesty. However, there are several possible explanations for these findings. One possible reason is that while the Honesty in Interpersonal Relationships and the Honesty/Integrity scales were both measuring the same trait (ostensibly, honesty), the Marlowe-Crowne measure of social desirability responding addresses a completely different phenomenon; that is, honesty and SDR are not related as had been assumed.

Another possibility involves the fact that honesty is a difficult concept to measure. Actual honest interactions were not definitively measured by any of the scales employed. Only "what was perceived to be true by the participants" and self-reported as such was available for analysis. "The definition of honesty was therefore left to the participants."[1b] What one individual may have considered honest may have been very different from another's idea of honesty, and therefore validity may have been compromised. It was essentially left to the individual to personally define "honesty," and as a result, the scales measuring honesty (Honesty in Interpersonal Relationships and Honesty/Integrity scales) may have made it much more salient to participants what was being assessed than the other scales.

Conversely, the Marlowe-Crowne scale, measuring SDR, a less salient form of dishonesty, may have facilitated participants answering more genuinely. Though a weak trend, the results for the Marlowe-Crowne scale (M = .3861) showed that individuals generally tended not to respond in a socially desirable manner, which suggests that they were in fact being honest. It is difficult to separate the implications of these results from those of the honesty measures. If individuals were not responding in a socially desirable way, this suggests that they were probably being honest in self-produced reports on the other scales. Additional research with different measures of honesty and SDR will be essential in further exploration of this relationship.

Because no statistical relationship between mindfulness and social desirability was found in the present study, this could be a topic of further research. Perhaps the use of a different scale of social desirability responding could help to further explore the relationship between mindfulness and various types of honesty. The Marlowe-Crowne scale, although widely used and well validated, may not tap into the aspects of social desirability that were the initial target of study. The Marlowe-Crowne scale is designed to focus on impression management, or the tailoring of one's behavior for an audience.[9] However, a different measure of SDR, the Balanced Inventory of Desirability Responding,[9] is divided into three subscales, assessing self-deception, impression management, and self-deceptive denial. Self-deception is especially of interest, both because of its association with a lack of awareness[10a] and because it is a much less salient form of dishonesty than the more overt measures present in some of the other scale. It was hypothesized that a mindful individual, by nature, would be more aware of himself, the present moment, and his experience and feelings about the present, and thus would be unlikely to employ self-deception. It is not clear that the Marlowe-Crowne scale properly tapped into this phenomenon. Although research analyses have indicated that the Marlowe-Crowne and BID-R measure the same construct (SDR), the partitioning of the different forms of social desirability may prove fruitful in getting at the true relationship between mindfulness and different forms of honesty and dishonesty.

This study has provided a valuable insight into an implication of mindfulness that had previously been unknown. This research has demonstrated the existence of a link between the quality of mindfulness and honesty, though much room is left for further exploration of this relationship. As individuals apply mindfulness to their lives, including how they perceive themselves and how they present themselves to and interact with others, we may be able to gain a deeper understanding of human nature. Though generally recognized as self-awareness, the concept of mindfulness could be applied to one's awareness of the reality of social interaction. By practicing mindfulness in their daily interactions, people could learn to be more honest and genuine both with themselves and with others.

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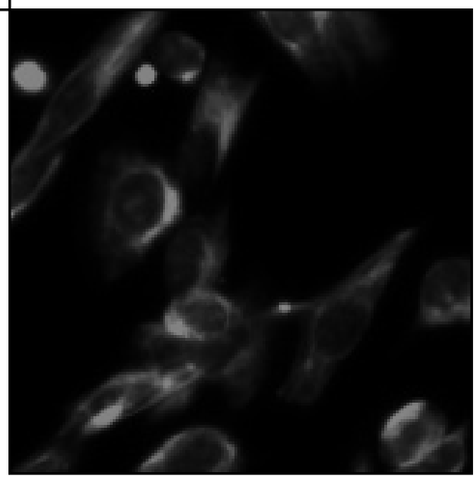
Kathryn Hefner is a senior Psychology major in the honors program, with a minor in Brain and Cognitive Science. The present study was conducted as part of a requirement for Research Methods of Psychology, under Dr. Kirk Warren Brown, who, along with Dr. Richard Ryan developed the MAAS scale used in this investigation. Kathryn is currently working with Dr. Jennifer Aube on her honors thesis, investigating the effects of exposure to parental marital conflict on adult attachment and trust in intimate relationships. After graduation, she hopes to return to the National Institute of Mental Health, where she interned during the summer of 2004. Upon completing this year-long post-baccalaureate fellowship in mental health research, she will pursue her doctorate in clinical psychology.

Josh Felver-Gant will graduate in 2005 with a degree in psychology. He will be working as a research assistant after graduation and plans to enter a PhD program in clinical psychology the following year.



Preliminary Evidence in Support of a Role for the AhR in Neural Development:
Implication for TCDD-Induced Neurotoxicity

Daniel T. Lioy, 2004

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Childhood exposure to environmental contaminants can lead to developmental abnormalities and pathologies later in life. One environmental pollutant implicated in the etiology of a range of developmental deficits and other biological impairments is the polyhalogenated aromatic hydrocarbon (PAH) congener 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[1,2] While TCDD is not deliberately manufactured today, it is an inadvertent byproduct of many industrial processes, including the incineration of various chlorine-containing wastes, the manufacturing of pesticides, and the bleaching of pulp and paper products.[3-9] In addition to its continued widespread production, TCDD is a highly stable molecule, with a half-life in the environment of up to 10 years.[10] Furthermore, due to its extremely low solubility in water and relative insusceptibility to metabolic breakdown, TCDD bioaccumulates in the adipose tissue of animals with high body fat to body mass ratios.[2,11-13] Thus, while there are varied incidents of mass exposure to high levels of TCDD in the human population through work-related accidents and the use of contaminated pesticides, the primary and more insidious mode of chronic human exposure to TCDD is through the consumption of animal products, especially dairy, beef, and fatty fish that are at the top of the food chain.[2,13-16]

Due to the prevalence and persistence of TCDD in the environment, risk factors associated with exposure have been the subject of major public health concern. Accidental TCDD exposure in humans elicits a variety of responses, including liver damage,[17, 18] endocrine disruption,[19, 20] carcinogenesis,[21,22] teratogenesis,[11,23,24] behavioral and cognitive perturbations,[20,25] and a severe skin condition known as chloracne.[26,27] However, widespread contact with high levels of TCDD in the human population is infrequent. Furthermore, exposure rarely occurs in isolation of other contaminants, making it difficult to attribute ensuing health problems exclusively to TCDD toxicity. To address this problem, animal models have been a useful tool in examining the effects of TCDD exposure and the underlying mechanisms by which TCDD exerts its effects.

Early exposure to TCDD in mice and rats leads to a broad spectrum of developmental abnormalities. Gestational exposure to TCDD has been linked to lowered birth weight and increased mortality rates in offspring.[23] Multiple organ systems also acquire altered developmental phenotypes following perinatal exposure to TCDD, including delayed tooth development,[28-30] delayed bone ossification,[31] hepatocyte atrophy,[32] thyroid hyperplasia and atrophy,[33] altered prostate and seminal vesicle development,[34,35] and delayed opening of the vaginal canal.[36] In post-natal mice, a single oral dose of TCDD decreases the long-term reconstitution activity of hematopoietic stem cells and alters the relative proportions of developing B-lymphocyte subpopulations in a dose-dependent manner.[37,38] Furthermore, treatment of proliferating keratinocytes in culture with as little as 1nM TCDD leads to an accelerated period of proliferation followed by the early onset of markers associated with epidermal keratinocyte differentiation.[37]

Early exposure to TCDD also leads to neurodevelopmental abnormalities at markedly lower exposure levels as compared to other tissues.[11] Male and female rats exposed to TCDD during critical periods of cortical development show a reversal of sex-specific cortical lateralization, with males acquiring left hemisphere dominance and females acquiring right hemisphere dominance.[39] Both male and female rats show an overall decrease in cortical thickness.[39] Prenatal TCDD exposure also abolishes sex-specific expression patterns of the γ-aminobutyric acid (GABA) synthesizing enzyme GAD67 in the preoptic area and anteroventral periventricular nucleus.[40] Furthermore, in the raphe nuclei of mice exposed to TCDD in utero and via lactation, a 50% decrease in serotonin-positive cells is seen as compared to control mice.[41] Taken together, these findings suggest that TCDD may act to deregulate points of control necessary for proper development in a variety of organ systems and cell types.

Although there may be several unidentified cellular targets for TCDD, the toxicological effects are most potently and primarily mediated by the aryl hydrocarbon receptor (AhR) signaling pathway.[42] AhR is an evolutionarily conserved ligand-activated transcription factor that belongs to a family of proteins containing basic-helix-loop-helix (bHLH) and PER-ARNT-SIM (PAS) homology domains.[43] Several studies point to a role for PAS proteins in the detection of changes in the extracellular environment and in the coordination of cellular responses to those changes. Consistent with this role for PAS proteins, AhR has been shown to mediate a pleiotropic response in multiple cell populations to coplanar xenobiotics, including many polychlorinated biphenyl and PAH congeners.[44,45] Of

these, TCDD binds to AhR with the highest affinity.[46] Prior to binding TCDD, AhR exists in the cytoplasm in a multimeric complex with the chaperone protein hsp90, the co-chaperone heat shock protein 23 (p23), and the Hepatitis B Virus X-associated protein 2 (XAP2).[47] Upon binding TCDD within the PAS domain, AhR dissociates from the complex and translocates to the nucleus where it forms a heterodimer with the AhR-nuclear-translocation protein (ARNT).[48] The AhR/ARNT complex then binds to xenobiotic-responsive enhancer elements (XREs) positioned upstream of target genes, which encode a battery of proteins including the CYP family of drug metabolizing proteins and the prostaglandin pathway enzyme cyclooxygenase-2 (COX-2).[49-51] After AhR/ARNT transcription of the targeted genes is completed, AhR dissociates from ARNT and undergoes a process of ubiquitination (Figure 1).[52,53]

While a role for AhR in the detection and in the mediation of cellular responses to environmental contaminants like TCDD is well-established, it is unlikely that this would be the sole function of AhR. The expression and highly conserved morphology of AhR across animal species is likely the result of a function uniquely fulfilled by AhR in the innate physiology of the cell. Indeed, several lines of evidence point to a role for AhR in regulating the activities of signaling cascades involved in cell-cycling and development.[54,55] Furthermore, the AhR pathway interacts with several steroid hormone signaling cascades, including the estrogen receptor, androgen receptor, and thyroid hormone receptor.[56,57] For instance, AhR binds to inhibitory XREs in the regulatory regions of ER-responsive genes.[54] A native role for AhR in cellular physiology is also supported by the discovery of endogenous ligands capable of activating the AhR pathway. Recent studies in yeast systems demonstrate that indirubin and indigo, two indol-containing molecules present in human urine, can bind to and activate the transcriptional activity of AhR at physiologically relevant levels.[58] Moreover, both indirubin and indigo have EC50 values comparable to or lower than that of TCDD, with indirubin being approximately fifty times more potent a ligand to AhR.[58]

In addition to mechanistic evidence in support of a dynamic role for AhR in native cellular processes, numerous studies implicate transcription factors that contain the bHLH-domain in the regulation of key pathways during development.[59,60]

Cellular development consists of three principle stages, including proliferation, determination, and differentiation, and the genetic programs of cells through each stage must be tightly orchestrated in order to achieve proper development of the whole organism.[61] bHLH-containing proteins have been shown to mediate the timing and passaging of a diverse range of cell populations through each developmental stage, particularly during neural development.[62,63] For instance, in corticogenesis the expression of the bHLH-proteins Id and Hes has been shown to maintain cortical progenitor cells in a proliferative state by antagonizing the activity of pro-neuronal bHLH-proteins and interacting with components of the cell-cycle machinery.[60,64,65] Conversely, a decrease in Id and Hes protein levels and an increase in the activity of bHLH-factor Mash promotes cell-cycle arrest and acquisition of a corticoneuron phenotype.[60,64] Interestingly, TCDD has been shown to induce the expression of Hes protein via an XRE positioned directly upstream of the hes-1 gene, suggesting that AhR may participate in the program of cell-cycle progression

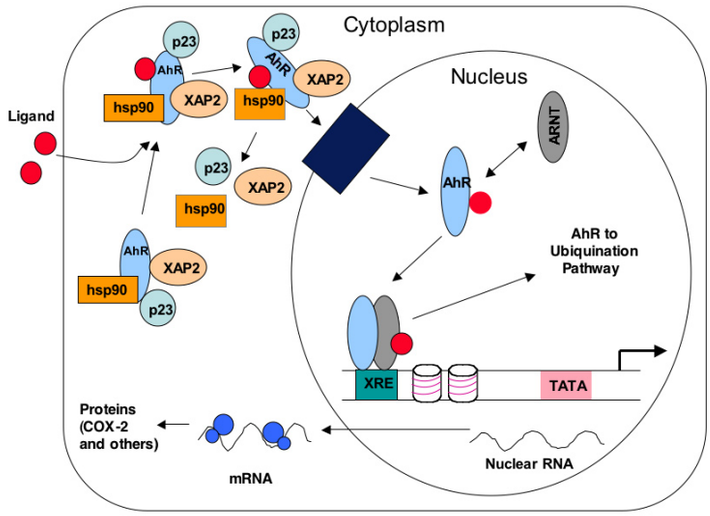

Figure 1: Cellular cascade showing the effects of TCDD on AhR.

and neural cell lineage determination.[66]

Similar patterns of regulation by bHLH-proteins in neurodevelopment are seen in cerebellar granule neurogenesis. In mammals, development of cerebellar granule neurons begins in the dorsal portion of the metencephalon known as the rhombic lip.[67] At embryonic day 13 (E13) in mice, a subpopulation of cells from the anterior rhombic lip migrate in a rostromedial direction over the surface of the developing cerebellar anlage to form the external granule cell layer (EGL). By E16, the EGL forms a thin envelope of rapidly dividing cells over the surface of the developing cerebellum. Between postnatal days 5 and 7 (P5 and P7), two distinct zones within the EGL are observable. The contributions of each layer to cerebrogenesis are different.[68] The outer EGL is a secondary zone of neurogenesis comprised of mitotic precursor cells while the inner EGL consists mainly of post-mitotic cells that express the cytoskeletal protein βIII-tubulin, an early marker of neuronal differentiation.[68,69] Cells in the outer EGL proliferate from approximately P0 to P15 with peak proliferation occurring from P3 to P10. After P10 the outer EGL begins to decrease in size as the cells migrate into the inner EGL. By P21, most EGL cells have migrated through the molecular layer and Purkinje cell layers towards the inner granule cell layer (IGL).[68] Once in the IGL, cerebellar granule neurons undergo terminal differentiation marked by the expression of the GABA receptor subunit α6.[70]

Two bHLH-proteins identified as critical to regulating this program of cerebellar granule neurogenesis are the transcription factors Math1 and NeuroD.[71,72] Math1 is the earliest known marker for cells in the rhombic lip that are destined for the EGL.[73,74] Studies using Math1-/- mice report a markedly reduced anterior rhombic lip and a lack of EGL formation, demonstrating an essential role for Math1 in the earliest stages of granule neuron development.[41] Conversely, distinct outer and inner EGL layers fail to form in animals genetically engineered to overexpress Math1. Markers of early granule neuron differentiation such as βIII-tubulin that localize to the inner EGL in wild type cerebellum are prematurely and diffusely expressed throughout the EGL in transgenic animals, suggesting that Math1 may also play a role in early granule neuron differentiation.[36] NeuroD also plays an essential role in granule neuron development as evidenced by a markedly

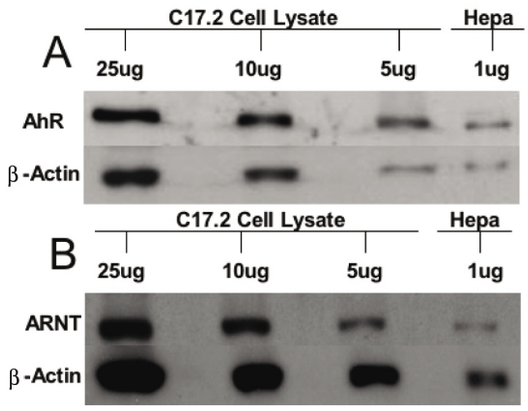

Figure 2: Immunoblot analysis of AhR (A) and ARNT (B) expression in C17.2 cells. 25ug, 10ug, and 5ug of protein from untreated C17.2 cell lysate were blotted for AhR and ARNT expression. Hepa1c1c7 cells were used as a positive control. Corresponding β-actin blots serve as loading controls. Results are representative of 2 separate experiments.

reduced IGL in NeuroD-/- mice.[72] Thus, bHLH-proteins are critical to the proper development of many cell types, including cerebellar granule neurons.

Interestingly, rat offspring exposed to TCDD during gestation exhibit alterations in behaviors associated with cerebellar function, including decreased performance on rotorod testing, increased motor reflexivity, and increased activity when placed in a novel environment.[63,64,113] Furthermore, the expression of AhR and ARNT mRNA in mature cerebellar neurons is exclusive to granule cells.[80] AhR and ARNT mRNA restriction to granule neurons in the cerebellum suggests a unique role for the AhR pathway in granule neuron physiology. However, despite mounting evidence in support of a role for AhR in the development of multiple cell populations, including the specification of GABAergic motorneuron subpopulaitons in C. elegans[38] and the effects of TCDD on behaviors which are contingent upon proper cerebrogenesis, a role for AhR in neurodevelopment remains largely unexplored. For instance, while it has been shown that AhR and ARNT mRNA are expressed in various brain regions, no studies to date characterize the expression of AhR and ARNT mRNA and the activity of the AhR pathway in the central nervous system (CNS). Furthermore, while previous studies have demonstrated that AhR and ARNT mRNA are expressed in the adult cerebellum, cerebellar dysfunction due to TCDD toxicity is associated with perinatal exposure.[113] If AhR is the primary mediator of TCDD toxicity and TCDD alters normal development of the cerebellum as evidenced by changes in behaviors that are dependent upon proper cerebrogenesis, then it stands to reason that AhR may play a role in development of the cerebellum. This line of reasoning, together with previous data from our lab demonstrating that cells isolated from the mouse EGL express AhR and ARNT proteins, raised the central hypothesis that the AhR pathway plays a role in the developmental program of cerebellar granule neurons.

This hypothesis was explored using the C17.2 cell line. C17.2 cells provide an attractive in vitro model for studying a role for AhR in cerebellar granule neurodevelopment for several reasons. Most importantly, C17.2 cells originate from the EGL,[48,105] a zone of rapidly proliferating precursor cells that, in addition to giving rise to terminally differentiated cerebellar granule neurons, may also give rise to other neural populations.[55,86,101] C17.2 cells maintain the ability to differentiate down several neural lineages as evidenced by studies in which C17.2 cells are grafted into various regions of the mouse and rat brains. C17.2 cells acquire the phenotypic markers of neurons and glia particular to the region of engraftment, including terminally differentiated granule neurons in the IGL.[59,67,89,117] Furthermore, lesioned animals that are implanted with C17.2 cells regain varying degrees of functionality associated with the region of neural ablation, suggesting that C17.2 cells also integrate functionally into local neural networks.[89] Thus, C17.2 cells represent a cell population that is in an early stage of neural development, a necessary property for the examination of a role for AhR in progressive stages of granule neuron maturation. Furthermore, the C17.2 cell line can be maintained in vitro in distinct developmental stages for prolonged periods of time, allowing for the direct examination of AhR activity in sequential phases of neural development.[48]

To gain additional insight into a potential role for AhR in neuronal maturation, studies were designed to evaluate the activity of the AhR pathway in C17.2 cells; more specifically to see whether C17.2 cells express a functional AhR pathway, and if so, whether AhR expression is differentially regulated during neuronal development. The findings of this study provide preliminary evidence in support of a role for AhR in neuronal maturation.

The C17.2 cell line was a generous gift of Dr. Evan Y. Snyder (Harvard Medical School). Briefly, the C17.2 cell line was originated from proliferating EGL cells of a P4 CD-1 x C57BL/6J hybrid mouse. Isolated cells were immortalized by retroviral transfection with the avian oncogene v-myc.[92] Cells were maintained either in serum-containing or serum-free medium referred to here as "feeding medium" and "defined medium," respectively. Protein concentrations of cell extracts were determined using a microBCA assay as specified by the manufacturer. All immunoblot and immunocytochemical analyses were performed using protein-specific primary antibodies complexed to biotinylated-secondary antibodies.

AhR and ARNT proteins are expressed in C17.2 cells

To determine whether C17.2 cells express a functional AhR pathway, C17.2 cell lysate was analyzed for AhR and ARNT protein content. C17.2 cells were grown in feeding medium and harvested two to three days after plating for immunoblot analysis. Both AhR and ARNT proteins were detected in C17.2 cell lysates. Hepa1c1c7 cell lysate served as a positive control. Corresponding b-actin blot confirms that decreasing AhR and ARNT signals correlate to decreasing amounts of total protein loaded (Figure 2).

AhR undergoes nuclear localization upon TCDD exposure

Previous studies report that in a variety of cell types, unbound AhR resides in the cytosol in a multimeric complex with hsp90, XAP2, and p23.[88] Upon binding to a ligand, AhR disassociates from the complex and undergoes translocation to the nucleus where it heterodimerizes with ARNT.[102] To determine the responsiveness of AhR to TCDD in the C17.2 cell line, cells were grown in feeding medium and fixed 5, 10, 15, 30, and 60-minutes following exposure to DMSO vehicle, 1nM TCDD, or 10nM TCDD. Immunocytochemical analysis revealed that in DMSO treated cells, AhR remained localized primarily to the cytosol, with minimal staining in the nucleus. Differences in the subcellular location of AhR were not observed in basal

conditions as compared to DMSO treated cells (data not shown). Conversely, in C17.2 cells treated with 1nM or 10nM TCDD, AhR staining was restricted mainly to the nucleus at all time points studied. Little difference was seen overall in the patterning of AhR staining between cells exposed to 1nM or 10nM TCDD. Hoechst staining of DNA confirmed the nuclear localization of AhR in TCDD treated cells (Figure 3). A live/dead assay indicated that exposure to DMSO vehicle, 1nM TCDD, or 10nM TCDD does not compromise the viability of C17.2 cells relative to basal conditions (data not shown).

AhR binds to XREs upon TCDD exposure

Upon binding an agonist, AhR translocates to the nucleus, heterodimerizes with ARNT, and the transcriptionally active AhR/ARNT complex then binds to XREs positioned upstream of target genes.[104] To resolve the XRE binding activity of AhR/ARNT in the C17.2 cell line, cells were grown in feeding medium and treated with DMSO, 1nM TCDD, or 10nM TCDD. Cells were harvested 1 hour following treatment for preparation of total cell extracts. EMSA analysis revealed no difference in the baseline levels of XRE binding in cells treated with DMSO vehicle as compared to basal conditions (data not shown). However, a 2.1 ± 0.5 and 2.5 ± 0.8 fold increase in AhR/ARNT-XRE binding was observed in cells treated with 1nM and 10nM TCDD, respectively, as compared to DMSO conditions. Unlabeled XRE-oligonucleotide band confirmed specificity of AhR/ARNT-XRE band (Figure 4).

AhR protein levels downregulate following TCDD exposure

Several reports demonstrate that AhR levels rapidly downregulate following ligand binding.[61,83] The downregulation of AhR appears to be ubiquitin-mediated as evidenced by studies in which inhibition of the 26S proteasome results in an attenuation of AhR protein depletion and enhanced AhR activity.[83] To determine if AhR levels downregulate in C17.2 cells exposed to ligand, cells were treated with DMSO vehicle, 1nM TCDD, or 10nM TCDD and harvested 4, 8, and 24 hours later for immunoblot analysis. In cells treated with DMSO vehicle, a pronounced signal from the AhR band was seen at all time points examined. Differences in AhR protein levels were not observed in DMSO treated cells as compared to basal conditions (data not shown). However, a marked reduction in AhR levels was observed in cells exposed to 1nM and 10nM TCDD as compared to DMSO treated cells at all time points examined. Corresponding b-actin blot is provided to confirm relative amounts of total protein loaded (Figure 5).

TCDD exposure induces the expression of COX-2 protein

The transcriptionally active AhR/ARNT heterodimer, once bound to the XRE of target genes, induces the expression of such proteins as COX-2, the first enzyme in the metabolic pathway that converts arachidonic acid to a group of molecules collectively known as eicosanoids.[50] To determine the ability of AhR in C17.2 cells to induce the expression of proteins known to be directly regulated by the AhR pathway, cells were treated with DMSO vehicle, 1nM TCDD, or 10nM TCDD and harvested 24 and 48 hours later for immunoblot analysis of COX-2 protein levels. Differences in COX-2 protein levels in DMSO treated cells versus basal conditions were not detected (data not shown). However, a 1.12 ± 0.09 and 1.16 ± 0.14 fold increase in COX-2 protein levels was observed in cells treated with 1nM or 10nM TCDD, respectively, as compared to DMSO conditions beginning 48 hours following exposure. In addition, AhR protein levels were downregulated in cells treated with 1nM and 10nM TCDD as compared to DMSO-treated cells, indicating that AhR-mediated signal transduction was altered upon TCDD exposure. Differences in COX-

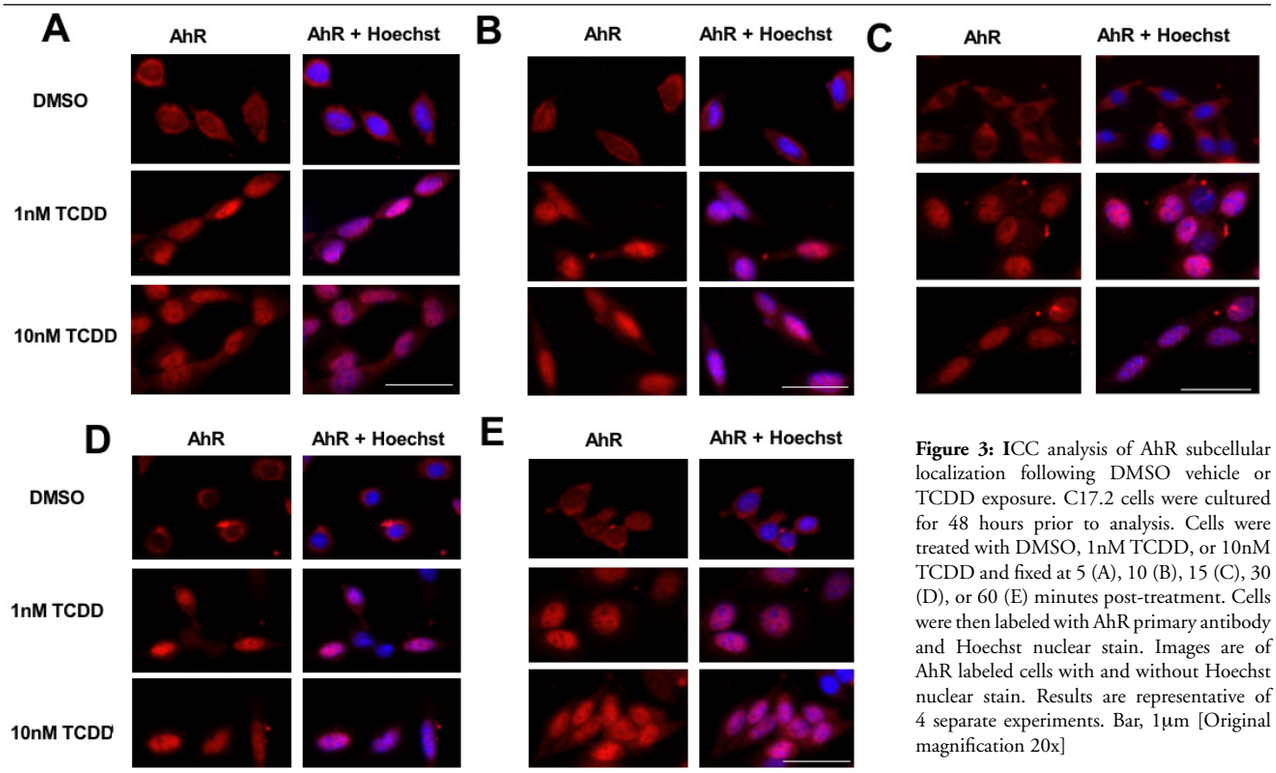

Figure 3: ICC analysis of AhR subcellular localization following DMSO vehicle or TCDD exposure. C17.2 cells were cultured for 48 hours prior to analysis. Cells were treated with DMSO, 1nM TCDD, or 10nM TCDD and fixed at 5 (A), 10 (B), 15 (C), 30 (D), or 60 (E) minutes post-treatment. Cells were then labeled with AhR primary antibody and Hoechst nuclear stain. Images are of AhR labeled cells with and without Hoechst nuclear stain. Results are representative of 4 separate experiments. Bar, 1μm [Original magnification 20x]

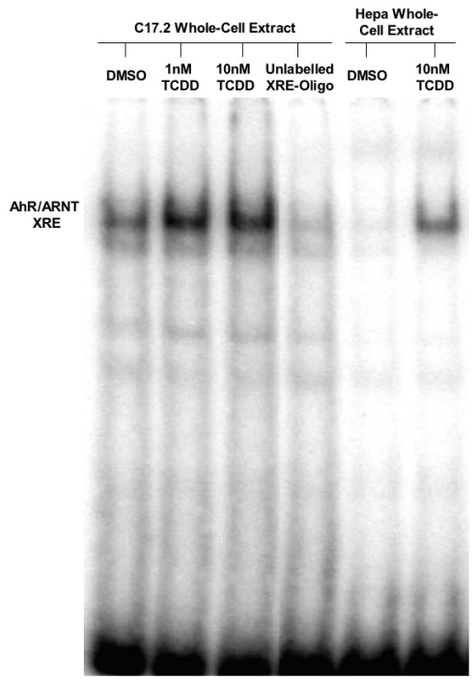

Figure 4: EMSA analysis of AhR/ARNT-XRE binding in C17.2 cells. Cells were maintained in feeding medium and treated with DMSO, 1nM TCDD, or 10nM TCDD 1-hour prior to whole-cell extraction. Lanes contain approximately 15ug of protein isolated from C17.2 cell nucleus. Whole-cell extracts from Hepa 1c1c7 cells treated with DMSO vehicle or 10nM TCDD were used as a positive control for XRE-binding. Unlabeled-XRE confirms specificity of AhR/ARNT-XRE band. Nuclear extracts Unlabeled-XRE band Results are representative of 3 separate experiments.

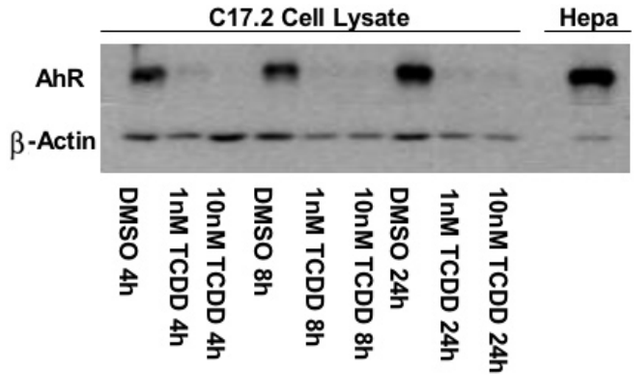

Figure 5: Immunoblot analysis of AhR (A) protein levels in DMSO vehicle or TCDD treated C17.2 cells. Cells were treated with DMSO vehicle, 1nM TCDD, or 10nM TCDD at 4, 8, or 24-hours prior to protein isolation. Lanes contain 10ug of protein isolated from C17.2 cells. Untreated Hepa1c1c7 cells were used as a positive control. Corresponding b-actin blot serves as a loading control. Results are representative of 3 separate experiments.

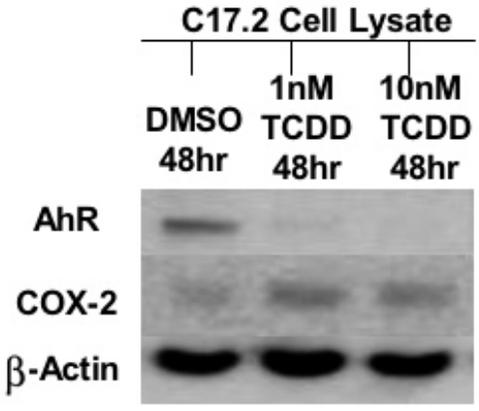

Figure 6: Immunoblot analysis of AhR and COX-2 protein levels in DMSO vehicle or TCDD treated C17.2 cells. C17.2 cells were treated with DMSO vehicle, 1nM TCDD, or 10nM TCDD at 48-hours prior to protein isolation. Lanes contain 25ug of protein isolated from C17.2 cells. Corresponding b-actin blot serves as a loading control. Results are representative of 3 separate experiments.

2 protein levels were not observed before 48 hours in cells treated with DMSO vehicle versus TCDD (data not shown). Corresponding b-actin blot is provided to confirm relative amounts of total protein loaded (Figure 6).

C17.2 cells can differentiate in a fully defined, serum-free medium into at least two morphologically distinct cell populations

When maintained in feeding medium, C17.2 cells rapidly proliferate as evidenced by their ability to grow to confluence in culture. Prior to reaching confluence, there are two general morphologies observable in the overall population of cells. Both subpopulations assume an irregularly shaped cytosolic domain, with a large nuclear compartment located near the center of the cell. However, one subpopulation of cells is flat and more rounded with multiple wide, flat, and short extensions protruding in all directions. A second subpopulation of cells acquires a more elongated morphology.

The standard feeding medium for C17.2 cells consists of a total of 15% serum (10% FBS and 5% HS). Serum contains a large number of unknown factors including proteins, peptides, cofactors, and other unidentified, biologically active compounds that are likely to have a profound influence on the physiology and morphology of C17.2 cells.[48] In order to identify a medium in which the factors that influence the developmental program of C17.2 cells in culture could be more precisely controlled for, cells were plated in the previously described and widely used Bottenstein and Sato medium, referred to here as defined medium.[11] Previous studies report that when maintained in this fully-defined medium, the C17.2 cell line remains viable for extended periods of time.[48] Furthermore, C17.2 cells maintained in defined medium have been shown to exit the cell-cycle, as evidenced by previous studies that report DNA content remains constant in C17.2 cells maintained in defined medium.[48]

To begin to define the differentiative capacity of C17.2 in culture, cells were plated in feeding medium for 24 hours and then replaced in defined medium. A subpopulation of cells maintained in defined medium alone spontaneously acquired a neuronal-like morphology beginning 4 days after treatment; the soma of a minority of cells became small and circular with 2 to 3 neurites of varying lengths protruding in opposite directions. The soma contains a large, centrally located nucleus and scanty cytoplasm. Furthermore, cells assuming a neuronal-like morphology tend to do so in clusters, leaving large expanses of the plate surface void of similarly appearing cells. The majority of cells however (approximately 70%) acquire morphology that is consistent with glial cells, maintaining a large, flat, and generally polygonal cytoplasmic domain with multiple large and flat protrusions. These cells also maintain a large, centrally located nucleus. Furthermore, cells acquiring the glial-like morphology appear to be spread evenly over the plate surface. The rate of C17.2 cell cycling in defined medium is markedly reduced relative to the rate of C17.2 cell-cycling in feeding medium, as evidenced by the inability of C17.2 cells to reach confluence when maintained in defined medium for as long as 14 days. After 7 days of maintenance in defined medium, the distinct morphology of the neuronal-like cells relative to that of the glial-like cells is more pronounced, insofar as the axonal-like processes are longer, thinner, and more highly networked.

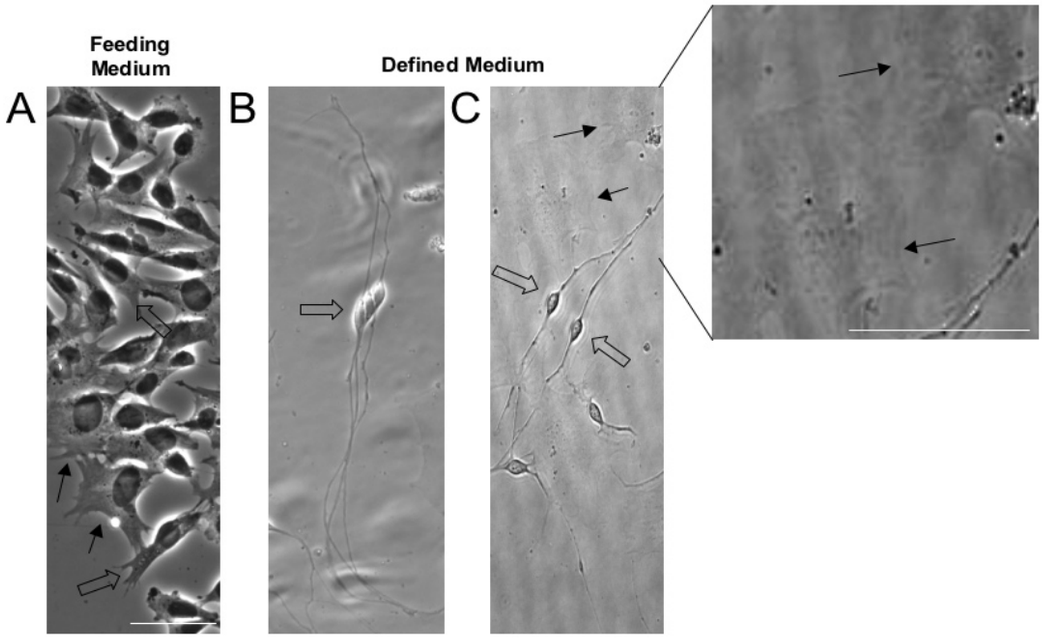

Figure 7: Morphology of C17.2 cells grown in feeding medium (A) or Bottenstein and Sato medium (B)(C) for 7-days. Cells grown in feeding medium maintain an irregular morphology with large nuclear and cytoplasmic domains. Some cells acquire a widened morphology with multiple protrusions (solid arrows) while other cells adopt a more elongated morphology (open arrows). Cells grown in defined medium for 7-days assume two distinct morphologies. A subset of cells develop axon-like, bipolar processes with a small, centrally located cell body that consists of a large nucleus and small cytoplasmic domain (open arrows). A separate subset of cells assume a flattened, glial-like morphology with a large, centrally located nucleus surrounded by a large cytoplasmic domain (solid arrows). Results are representative of 9 separate experiments. Bar, 1μm. [original magnification 20x]

The figure provided depicts the morphology of C17.2 cells grown in defined medium for 7 days (Figure 7).

C17.2 cells undergo neuronal differentiation in culture

Prior to differentiating toward a neuronal or glial lineage, proliferating neural precursor cells express the intermediate filament nestin, a marker that is universally expressed in dividing neural stem cells.[54,58,78] Upon cell-cycle arrest, individual neural precursors can differentiate toward a neuronal or glial cell fate.[81] Cells pushed to actively differentiate down a neuronal lineage will express the microtubule subunit βIII-tubulin, an early marker of neuronal differentiation.[12] Similarly, cells pushed to actively differentiate down a glial lineage will express GFAP, an early marker of glial differentiation.[24,68] To further characterize the differentiative capacity and phenotypic traits of C17.2 cells in culture, cells were stained for nestin, βIII-tubulin, and GFAP proteins. Co-labeling with Hoechst nuclear stain revealed that when grown in feeding medium for 4 days, all cells express nestin protein, but not βIII-tubulin or GFAP proteins. While a majority of cells (approximately 95%) maintain nestin expression when kept in defined medium for 4 days, a minority of cells gain expression of βIII-tubulin protein. C17.2 cells that show expression of βIII-tubulin protein also exhibit morphology that is consistent with a neuronal phenotype (Figure 8). Cells did not, however, acquire expression of GFAP protein (data not shown). These data demonstrate that individual C17.2 cells can spontaneously differentiate in defined medium from a pluripotent neural precursor cell into a post-mitotic neuronal cell.

The expression of AhR protein is downregulated in post-mitotic C17.2 cells

To evaluate a role for AhR in neuronal development, AhR protein levels were assessed in C17.2 cells at sequential stages of neuronal development. C17.2 cells were grown in feeding medium or defined medium and harvested after 2 to 6 days for immunoblot analysis of AhR protein content. A marked decrease in AhR protein levels was observed at all time points in cells maintained in defined medium as compared to those maintained in feeding medium. In contrast, β-actin protein levels increased in cells maintained in defined medium. AhR levels were normalized to the corresponding β-actin blot (Figure 9).

Because of the heterogeneous expression of βIII-tubulin protein in cells maintained in defined medium, it was necessary to determine whether AhR protein downregulation is the result of a uniform downregulation in post-mitotic C17.2 cells or is the result of downregulation in a subpopulation of cells. Cells were kept in feeding medium or defined medium and fixed after 6 days in culture. Immunocytochemical analysis revealed that AhR protein is downregulated uniformly in all cells maintained in defined medium. Cells maintained in feeding medium served as a positive control (Figure 10). These data demonstrate that AhR protein levels are uniformly downregulated in non-dividing C17.2 cells as compared to AhR protein levels in mitotic C17.2 cells.

TCDD does not alter the proportion of C17.2 cells that express βIII-tubulin protein

Previous studies have demonstrated in several cell populations that TCDD can alter the expression of markers associated with distinct phases of cellular development.[40,87,110] To determine whether the developmental program of C17.2 cells is altered upon exposure to TCDD, cells were treated with DMSO vehicle, 1nM TCDD, or 10nM TCDD following a 4 hour incubation in defined medium. Cells were fixed 6 days later for immunocytochemical analysis of βIII-tubulin expression. Differences in the percentage of βIII-tubulin positive cells present in untreated cultures or those treated with DMSO vehicle were not detected (data not shown). Similarly, differences were not detected after TCDD treatment (Table 1). Furthermore, the number of βIII-tubulin positive cells in samples treated with 1nM or 10nM TCDD were well within the range of C17.2 cells that normally express βIII-tubulin protein after 6 days in defined medium (approximately 25 to

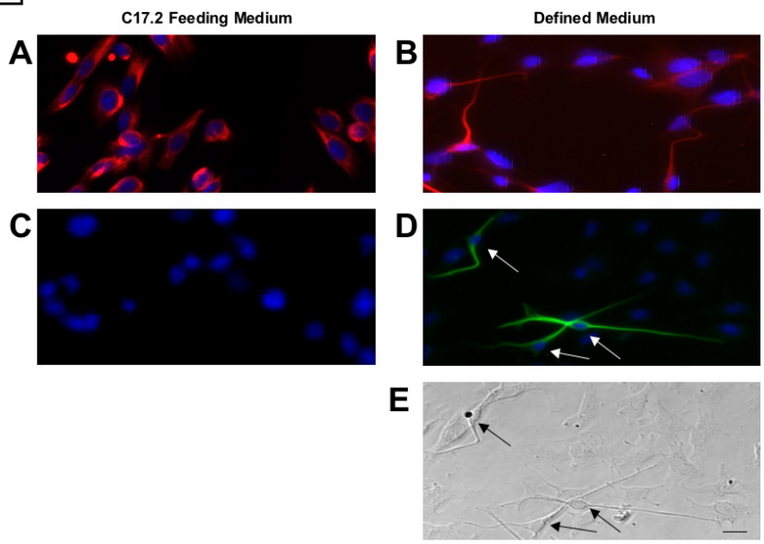

Figure 8: ICC analysis of nestin protein (A)(B) and βIII-tubulin protein (C)(D) expressions in C17.2 cells maintained in feeding medium (A)(C) or D.M. for 4-days (B)(D). Cells were fixed and then labeled with nestin or βIII-tubulin primary antibodies and Hoechst nuclear stain. A phase contrast image of field D is provided (E). Arrows designate corresponding βIII-tubulin positive cells in fields E and D. Results are representative of 3 separate experiments. Bar, 1μm. [original magnification 20x]

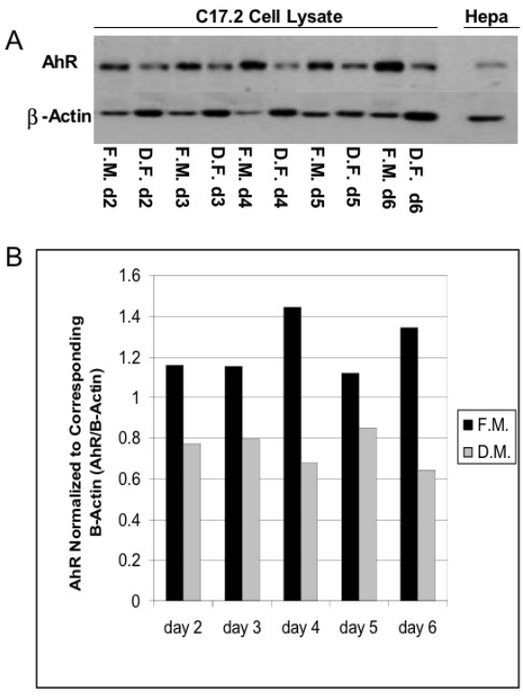

Figure 9: AhR protein content in C17.2 cells grown in F.M. or D.M. Immunoblot analyses of AhR protein levels in C17.2 cells maintained in F.M. or D.M. for 2 to 6-days. Lanes contain 10ug of protein isolated from C17.2 cells. Untreated Hepa1c1c7 cells were used as a positive control. Corresponding _-actin blot is provided (A). Bar chart of AhR protein levels normalized to _-actin is provided. Results were generated with Scion Image software (B).

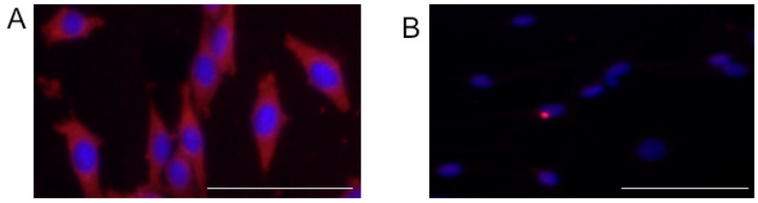

Figure 10: Immunocytochemical analysis of AhR protein distribution in C17.2 cells maintained in F.M. (A) or D.M. (B) for 6-days. Cells were fixed and then labeled with AhR primary antibody and Hoechst nuclear stain. Bar, 1μM. [original magnification 20x] Results are representative of 2 separate experiments.

32%). These data demonstrate that when maintained in defined medium alone, TCDD does not alter the relative proportion of C17.2 cells that express βIII-tubulin protein.

This study demonstrated that C17.2 cells contain a TCDD-responsive AhR pathway. Importantly, C17.2 cells were shown to express AhR protein and its dimerization partner ARNT, the two primary components requisite to initiate the AhR signaling cascade. Upon C17.2 cell exposure to TCDD, AhR underwent nuclear localization 5 minutes after treatment and bound to XREs positioned upstream of target genes. Furthermore, AhR protein was downregulated in samples treated with TCDD, demonstrating an alteration in AhR-mediated cell signaling.

Consistent with previous studies that have identified the cox-2 gene as a direct target of the AhR pathway in canine kidney cells, 50 changes in COX-2 protein levels following C17.2 cell exposure to TCDD were detected beginning 48 hours after treatment. COX-2 protein expression is first seen in canine kidney cells beginning 4 hours following activation of the AhR pathway.[49] Therefore, the cox-2 gene may not be a direct target of the AhR pathway in C17.2 cells. AhR may indirectly mediate the levels of COX-2 protein by directly regulating the expression of transcription factors that in turn bind to response elements positioned immediately upstream of the cox-2 gene. Alternatively, the AhR pathway may directly induce low level expression of COX-2 protein, although the assay used may not be sensitive enough to detect small changes in COX-2 protein levels. Low level induction of COX-2 protein may in turn initiate a feedback loop in which expression of the cox-2 gene is positively regulated, resulting in a delayed increase of detectable levels of COX-2 protein.[100] Therefore, the cox-2 gene may be a direct target of the AhR pathway in C17.2 cells, while the delayed increase in detectable COX-2 protein levels may result from the additive effects of a positive-feedback loop.

It is also possible that TCDD treatment affects the levels of COX-2 protein in C17.2 cells by an as-yet unidentified, AhR-independent mechanism. It has been shown that COX-2 is degraded by the ubiquitination pathway.[101] Therefore, TCDD may promote an increase in COX-2 protein by inhibiting its ubiquitination. To date TCDD has only been shown to increase the turnover of proteins, although it is possible that TCDD could play a role in decreasing the ubiquitination of proteins and thereby stabilizing an increase in protein levels.[102, 103] The development of reliable, specific inhibitors to AhR will help to determine conclusively whether upregulation of COX-2 protein in C17.2 cells exposed to TCDD is the result of an AhR-mediated event.

This study also demonstrated that individual C17.2 cells retain the intrinsic capacity of cerebellar EGL cells to differentiate towards a neuronal cell fate. When grown in feeding medium, C17.2 cells represent a pluripotent population of neural precursor cells as evidenced by the expression of nestin protein, a marker universally expressed by dividing neural stem cells.[92] C17.2 cells readily proliferate when maintained in feeding medium as determined by the ability of C17.2 cells to reach confluence. Previous studies have also shown that the DNA content in C17.2 cells increases with time in culture when incubated in feeding medium, indicating that cells are dividing.[80] In contrast, defined medium lacks the appropriate mitogenic factors to maintain C17.2 cells in a proliferative state. C17.2 cells cultured in defined medium thus fail to reach

confluence and, as previously reported, the DNA content remains constant over time.[80] Furthermore, a subpopulation of C17.2 cells differentiates into post-mitotic, neuronal-like cells as evidenced by the appearance of βIII-tubulin protein beginning 4 days after plating in defined medium. While a subpopulation of C17.2 cells assume a neuronal morphology and acquire expression of βIII-tubulin, the majority of C17.2 cells assume a glial-like morphology with a large, flat, and irregularly-shaped cytosolic domain and large nucleus. Based on morphological analysis and a lack of βIII-tubulin staining in these cells, it is unlikely that this subpopulation is developing toward a neuronal lineage. While C17.2 cells were devoid of GFAP protein expression, previous studies have reported that the majority of C17.2 cells plated in defined medium acquire GFAP staining, suggesting that this subpopulation may be fated for an astrocyte lineage.[86,104]

Fate decision processes are mediated by a complex set of extracellular and intracellular factors that together alter the genetic program of a cell in order to produce a novel regulatory state.[105] The passaging of cells through successive regulatory states ultimately leads to lineage commitment and differentiation. It is likely that C17.2 cell expression of GFAP protein is dependent upon the balance and timing of multiple extracellular factors, including trophic factors and cell-cell contact. Without the appropriate cellular environment, C17.2 cells may not acquire the expression of GFAP protein. Cells assuming a glial-like morphology may be fated for a glial lineage, while not yet achieving a regulatory state in which GFAP protein is expressed. Alternatively, cells that acquire morphology consistent with glial cells may not yet be committed to a glial or neuronal state, as evidenced by the continued expression of nestin protein and lack of βIII-tubulin and GFAP proteins. Supplementing defined media with trophic factors known to promote a neuronal lineage, such as the brain-derived neurotrophic factor (BDNF),[106,107] may help to determine whether cells that are devoid of GFAP and βIII-tubulin staining are committed to a glial lineage or maintain the capacity to differentiate towards a neuronal fate.

Interestingly, AhR protein levels are differentially regulated in proliferating and non-dividing C17.2 cells. AhR protein declines in C17.2 cells that are maintained in defined medium as compared to feeding medium, suggesting that AhR levels are downregulated in non-dividing cells relative to proliferating cells. Non-proliferating populations are not, however, devoid of AhR protein content. While AhR protein levels are reduced, immunoblot analysis confirmed the presence of AhR protein in cells maintained in defined medium. Sustained expression of AhR in non-dividing neural populations may reflect a function for AhR in mediating adaptive cellular responses to environmental contaminants, which is consistent with its role in detection and mediation of cellular responses to environmental factors.[44] Similarly, the continued expression of AhR in non-dividing C17.2 cells could reflect a role for AhR in intercellular communication processes.[108] In support of this possibility, AhR has been shown to interact with several extracellular signaling molecules including hormone-receptor pathways[54] and circulating indigoids.[109] AhR may regulate physiological processes dependent upon these extracellular factors in neural cells.

While this study did not aim to define a finite role for AhR in neural development, the findings suggest that AhR may participate in the transition from proliferation to cell-cycle arrest in C17.2 cells. AhR protein is expressed in all cells when maintained in feeding medium and is downregulated uniformly in cells plated in defined medium. However, despite the continued uniform expression patterns of AhR protein in C17.2 cells, the non-proliferating cell population is comprised of at least two distinct cell types. This suggests that AhR may not function in the determination of lineage commitment or the differentiation process of a particular subpopulation of neural cells. In light of the synchrony between AhR protein downregulation and the transition of C17.2 cells from proliferation to cell-cycle arrest, it is more likely that AhR may function to regulate components of the cell-cycle machinery in C17.2 cells. Therefore, AhR may aid in neurodevelopment by modulating the proliferation of neural precursor cells, since cell-cycle arrest is a prerequisite to neuronal differentiation.

	Percentage of bIII-tubulin Positive Cells ± S.E.M.
DMSO Vehicle	29.40 ± 1.48%
1 nM TCDD	29.59 ± 1.91%
10 nM TCDD	30.61 ± 2.12%

Table 1. The number of C17.2 cells that express βIII-tubulin protein after 6-days of exposure to DMSO vehicle, 1nM TCDD, or 10nM TCDD in defined medium. Cell counts are represented as the percentage of βIII-tubulin positive cells relative to the overall population ± S.E.M. Results represent 4 separate populations of cells per treatment group within one experiment.

In support of a role for AhR in regulating the program of cell-cycle progression, previous studies have identified the gene encoding the p27kip1 cyclin/cyclin-dependent kinase inhibitor as a direct target of the AhR pathway.[110] AhR has also been shown to mediate the transcription of the c-myc gene, a potent proliferative factor, by forming a transcriptionally active complex with the NF-KB subunit RelA.[111] Interestingly, COX-2 activity has also been implicated in mediating the program of cell-cycle progression in a variety of cell types.[44,112] While this study does not provide direct evidence in support of COX-2 as a mediator of cell-cycle progression in C17.2 cells, it demonstrates that COX-2 protein levels are increased in cells exposed to TCDD, possibly by an AhR-mediated event. It has also been previously shown that TCDD alters the transition from proliferation to cell-cycle arrest in a variety of cell populations via the AhR pathway.[113] The ability of TCDD to alter the program of cell-cycle progression is likely the result of a pleiotropic response directed in part by the AhR pathway.[114] AhR-mediated alterations in COX-2 activity in cells exposed to TCDD may be one component of this complex genetic response.[115-117]

Despite the expression of a TCDD-responsive AhR pathway in C17.2 cells and increased levels of COX-2 protein, TCDD did not produce detectable alterations in C17.2 cellular development. The absence of observable effects on C17.2 development in response to TCDD treatment could be a result of the experimental design as opposed to a lack of influence of TCDD on C17.2 cell differentiation. In this study, the percentage of βIII-tubulin positive cells was compared between untreated and TCDD treated C17.2 cell populations after 6 days

of exposure in defined medium. If TCDD alters the timing of C17.2 cell progression from the proliferative phase of cellular development to the cell-cycle arrest phase as it has been shown in keratinocytes,[37] then the proportion of cells expressing βIII-tubulin may be altered as a result of a prolonged or shortened proliferation phase. However, defined medium alone is a potent inhibitor of cell-cycle progression.[80] Consequently, any influence TCDD may exert on the transition of C17.2 cells from proliferation to cell-cycle arrest may be masked by the anti-mitogenic effects of the media. To determine whether TCDD alters the developmental program of C17.2 cells, the anti-proliferative effects of the defined medium will likely have to be balanced with the activity of a mitogenic factor. The development of culture conditions in which C17.2 cells can be maintained in a state of proliferation by factors controlled for by the experimenter will allow for an accurate assessment of the effects of TCDD on the developmental program of C17.2 cells. Previous studies have demonstrated that C17.2 cells can be made to proliferate in defined medium by the application of epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF).[80] Culturing C17.2 cells in defined medium supplemented with EGF or bFGF may allow for the detection of an alteration in the program of C17.2 cell-cycle progression and development upon exposure to TCDD.

This study showed that C17.2 cells express a functional AhR pathway at an early stage of neural development and that AhR protein levels are differentially regulated according to the phase of cell-cycle progression and development. This, in combination with the finding that individual C17.2 cells maintain the capacity of EGL cells to differentiate into neuronal-like cells, supports the notion that the C17.2 cell line can serve as a relevant in vitro model of AhR involvement in normal neurodevelopment. The generation of AhR knockout cells in combination with specific inhibitors to AhR will help to address the question of whether AhR is necessary to the developmental program of C17.2 cells. Furthermore, the generation of culture conditions in which whole populations of C17.2 cells can be guided predominantly towards a neuronal or glial fate will allow for the direct analysis of AhR activity during the development of specific neural lineages.

This study also provided preliminary evidence suggesting that TCDD does not alter the developmental program of C17.2 cells in defined medium. Supplementing the defined medium with mitogenic factors to C17.2 cells will allow for a more accurate assessment of whether TCDD alters C17.2 cell development. However, based on the expression of a functional AhR pathway in C17.2 cells, this study supports the notion that neural precursor populations may be direct targets of TCDD-mediated developmental toxicity.

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Agency, Depression, and Social Support in Residents of a Domestic Violence Shelter

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Intimate partner violence (IPV) is a pervasive and persistent social problem that requires attention and further study for us to understand its impact on American homes and communities.[1,2] IPV can manifest itself through physical, emotional, sexual, financial, and psychological abuse. This threat is particularly concerning for women, as seen in a recent study of the National Institute of Justice and the Centers for Disease Control and Prevention. In this study of sixteen thousand participants, 25.5% of women reported experiencing rape, physical assault, or stalking by an intimate partner at some point in their lives, while 7.9% of men reported such experiences3. Another study by the United States Department of Justice found that violence against women is primarily intimate partner violence: 76% of women surveyed who had been raped and/or physically assaulted since age 18 reported assault by a current or former husband, cohabiting partner, or date, compared with 18% of men.[3] One study found that IPV towards women is so prevalent that an adult female is more likely to be a victim of violent crime by her male partner at home than anywhere else or by anyone else.[4] Such significant statistics point to the importance of further study to better understand violent behavior in a domestic setting, as well as its antecedents and future implications. Domestic abuse is often severe and pervasive, resulting in physical injuries, numerous physical health problems,[5-7] mental health issues,[5,8-11] and sometimes death.[1,12,13] Such severe outcomes impact the whole of a woman's functioning, and the implications pervade into other spheres of her life.

Violent domestic relationships have a considerable impact on the mental health of those experiencing the abuse. Depression is a particularly concerning implication of engaging in an abusive relationship. One study found that in a sample of 110 abused women, 65% of them reported clinically significant levels of major depression.[14] Previous research has also found that abused women present more depressive symptoms than other women.[15]

Individuals who are depressed often feel worthless, fearful, guilty, and powerless to control their situations, while those with more severe cases of depression may consider suicide.[16] Abused women tend to have low self-esteem and often consider themselves failures as partners and peacemakers; some consider suicide as a way to end such feelings.[17] Post-traumatic stress disorder, major depression, alcohol abuse and dependence, and avoidant personality are more prevalent among abuse survivors than in a control group.[14]

Furthermore, people are particularly vulnerable to depression when they have poor social support.[18] Therefore, it is important to look at social support as a protective factor in regard to depression. The concept of social support includes an umbrella of various constructs such as support network resources, supportive behaviors, and subjective appraisals of support.[13,19-22] Some researchers have also discerned different modes of social support.[22] Emotional support involves behaviors such as listening, encouraging, and showing sympathy, while practical support involves behaviors such as providing monetary assistance, child-care, and transportation. Both forms of support have been found to relate positively to well-being. Previous research has found that the presence of a social network reduces mortality,[23] enhances psychological well-being, and protects against the distress that can accompany negative life events.[24] Social support also serves as a protective factor against burnout,[25,26] which is defined as a state of emotional and cognitive exhaustion caused by investment in emotionally taxing situations.[27] Social support, particularly from sympathetic and understanding people, improves overall health and increases life expectancy.[28]

Social support may be a particularly important factor for women in abusive relationships. Abusers often isolate their victims from interpersonal contact with others, preventing the victim from realizing that their relationship may be unhealthy.[29] Perpetrators of IPV often use social isolation to effectively control and assault women with less fear of detection.[12,30] Abused women often experience psychological entrapment and develop symptoms of learned helplessness,[31] which may prevent them from reaching out to others for help. Women in abusive relationships often state that their ability to communicate with family and friends had been seriously truncated and that they feel they had no one to turn to for help.[32] Social support is an important outlet, as women report that receiving help from family or friends is helpful in their ability to leave their abusive relationship.[33] However, few studies have directly examined the role that social support may play in protecting abused women from experiencing depression. It is likely that victims of domestic violence will experience higher levels of

depression if they do not have the social support of their family and friends, both in terms of practical support, which can be critical for women attempting to leave an abusive relationship, and of emotional support. Thus, the first goal of the study is to examine the role of social support in depression among domestic violence survivors.

Alongside social support, women are often in need of more community services to assist in building a healthy life. Resources such as medical care,[34] child-care,[35] safe and affordable housing,[32] and social service assistance[34] are often necessary for women to become independent. Communities with organized social institutions to respond to issues of IPV have had successes.[35,36]

One such community institution is a domestic violence shelter, which provides a safe haven for individuals who are looking to escape the threat of an abusive partner. Domestic violence shelters often offer services including individual and group counseling, networking with social agencies for funds and employment, and contacts with landlords for secure and reasonably priced housing. Through contact with both residents and staff in a domestic violence shelter, a new resident has the opportunity to develop a safe social support system outside of her previous abusive relationship.

However, previous studies have not examined the relation between women's utilization of shelter-based support systems and women's experiences of depression. Extrapolating from the general research on social support and mental health, it seems reasonable to postulate that women who are willing to rely on shelter staff and residents for social support may experience less depression during their shelter stay than women who are unable or unwilling to do so. The second goal of the study, therefore, is to examine the relationship between experiences of support in the shelter and depression levels at discharge.

If social support and networking have a positive impact on a woman's mental health, it is critical to look more closely at the ways in which social support can be established and maintained in the lives of survivors of IPV. Even under very difficult circumstances, such as with an abusive and controlling partner, some women are better able than others to seek out and experience social support from family and friends. Identifying these individual difference variables may be an important step in better understanding the relationship between social support and mental health in abuse survivors.

One such variable is agency. Agency is a term defined to reflect a fundamental modality of human existence as an individual,[37] and reflects an orientation towards the self: self-assertion, self-expansion, self-control, and self-direction.[38] Agency is linked to gender, in that men tend to have higher levels than women because typical gender-socialization experiences emphasize the development of these qualities in males.

In contrast, gender socialization processes are more likely to emphasize communion, or the fostering of connections, with females.[37] Previous research indicates that agency is also present in women and it has a pervasive and positive influence on psychological well-being for both men and women.[38] Agency is associated with reduced depression,[39,40] reduced anxiety,[40] enhanced self-esteem,[41] and reduced distress.[42]

Previous research has also indicated that social support is positively related to agency.[37] For individuals who are high in agentic qualities, high social support serves as a buffer against stress.[43] Agency has also been connected with help-seeking behavior.[37] This is consistent with research showing that women who are employed and less economically dependent are more likely to bring assault charges against their partner, obtain a protection order, and finally leave their abusive partner.[31] Protective factors such as employment and economic independence are agentic in nature. Thus, it may be the case that women with higher levels of agency are more willing or able to seek out and accept social support, lowering their levels of depression. The third goal of the study is to examine the hypotheses that women with higher levels of agency will report greater social support from both people outside the shelter and inside the shelter, and that this support will result in lower levels of depression upon leaving shelter.

In summary, it is clear that domestic violence is a serious and pervading issue for both individuals and the wider community. Implications of abusive relationships impact women in notable ways, and many women who have been abused experience increased levels of depression. Additionally, women who have been abused often experience isolation, which may disconnect them from social support, further increasing their levels of depression. However, women with agentic traits may be more likely to seek out and receive social support even under these difficult conditions and thereby experience lower levels of depression. Therefore, it is reasonable to speculate that social support may be a mediator in the relationship between depression and agency. Understanding the relation between these variables in this sample is important for being able to provide effective services for women in need.

Present Study

This study examined the relationship between agency, social support, and depression in a sample of female residents at a domestic violence shelter. Residents were assessed within one week of entering shelter (Time 1) and at a second appointment, within one week of their discharge from shelter (Time 2). Agency may be a contributing factor for abused women in the development of social support, both inside and outside of shelter life. This study hypothesized that abused women high in agentic traits at Time 1 would present lower levels of Time 2 depression, controlling for their initial depression level. In other words, women who came to shelter with high levels of agency would exhibit lower levels of depressive symptoms at the time of their discharge. Furthermore, social support of family and friends at Time 1 would be associated with lower levels of depression at Time 2, and social support in women's lives outside of shelter was predicted to be a mediator in the relationship between agency and depression. Finally, willingness to rely on the support of staff in the shelter was also predicted to be a mediator of the relationship between agency and depression.

Participants

Location: The participants of this study were residents of a domestic violence shelter for women and children located in southeastern Michigan. This study recruited a sample of fifty-eight abused women between the ages of 19 and 59, with a mean age of 33.42.

Racial Profile: 56.9% African American, 22.4% Caucasian, 5.2% Asian or Pacific Islander, 3.4% American Indian; 8.6%

of participants did not identify with any of these groups.

Education levels: 43.1% completed or attended some college, 50% completed high school or a general equivalency degree, and 5.2% completed only junior high school.

Abusive individual: All participants had recently (in the past seven days) been involved in an abusive relationship in a domestic setting and had been admitted to the shelter due to the imminent danger of further violence in their relationship. Most participants' (63.8%) abusive relationships were with a boyfriend, while 24.1% of participants were abused by a husband, and 10.3% had an abuser that was neither their boyfriend nor their husband.

Time of involvement with abusive individual: 58.6% of the women had been involved with the abusive individual for over one year, 24.1% had been with their abuser between six months and one year, and 15.5% had been involved with their abuser for less than six months.

Method

Each study participant was invited to complete paper and pencil measures on two separate occasions: once at a set time within a week of their arrival at the shelter (Time 1), and once during a set time within a week prior to their discharge (Time 2). Average time between initial assessment and subsequent follow-up was approximately three and a half weeks. The women were offered monetary compensation of a total of ten dollars for their participation; five dollars for each appointment. Average length of time of each appointment was approximately thirty to forty-five minutes. Each appointment was conducted in a quiet room away from the other shelter residents. An occupancy sign was posted on the outside door of the room for the entirety of the appointment, to allow for the greatest privacy possible. Of the initial sample of 58 women, only 22 participated in the follow-up session, despite efforts to follow-up with each woman. Analyses indicated that the women who completed the Time 2 data did not differ from the women who failed to participate at Time 2, on measures of depression, agency, or social support assessed at Time 1.

Participants were requested to complete measures about demographic information, depression (Center for Epidemiological Studies Depression Scale, CES-D), gender-related personality traits (The Extended Personal Attributes Questionnaire, EPAQ), social support (Social Support Behaviors Scale, SSBS), and emotional reliance. The CES-D was used both at Time 1 and Time 2, the EPAQ was used only at Time 1, the SSBS was used only at Time 1, and the ERQ was used only at Time 2.

Results

Table 1 presents the means and standard deviations for all study variables. Table 2 presents the Pearson correlations among the variables. Analyses involving depression at Time 2 included only 22 of the original 58 women who were included in the Time 1 analyses. Results showed that agency was positively associated with perceived social support from both family ($r=.32$, $p<.05$) and friends ($r=.39$, $p<.05$). Although not reaching significance, there was a trend indicating that agency, assessed at Time 1, was also associated with greater willingness to rely on shelter staff at Time, and with less depressive symptoms at follow-up. Social support received from friends at Time 1 was negatively related to Time 2 depressive symptoms ($r=-.46$, $p<.05$), and willingness to rely on shelter staff was marginally negatively related to depressive symptoms ($r=-.37$, $p<.10$). Perceived social support from family and friends at Time 1 was also positively correlated with women's willingness to rely on shelter staff at Time 2 ($r=.45$, $p<.05$ and $r=.41$, $p<.05$, respectively).

Because the relation between agency and Time 2 depressive symptoms did not reach significance, an indirect relationship between agency and depressive symptoms was hypothesized to exist through the woman having: (a) greater social support from friends; and (b) greater willingness to rely on shelter staff for support.

In order to test the hypothesis that agency was associated with decreased levels of depression through a subject's higher level of social support from friends, the variables were first mean-centered, and two hierarchical regressions were employed. First, the social support variable was regressed onto agency, after first entering Time 1 depression. Second, Time 2 depression was regressed onto social support, while holding agency and Time 1 depression constant by entering them first. The resulting unstandardized path coefficients and standard errors were used in the equation to assess the significance of the indirect relationship between agency and depressive symptoms through friends' social support. The indirect relationship was significant ($z'=1.49$, $p<.01$). The model accounted for 24% of the variance in Time 2 depressive symptoms ($F(3,17) =3.12$, $p<.05$).

It was also hypothesized that agency would result in less Time 2 depression through greater willingness to rely on shelter staff for support. Following the steps outlined above, a significant indirect relationship between agency and less depression was found through the pathway of greater willingness to rely on shelter staff ($z'=.94$, $p<.05$). Figure 1 depicts the results of the mediational analyses.

Analysis

The first goal of the study was to examine the role of social support in depression among domestic violence survivors. Results showed that there was a significant relation between social support at Time 1 and decreased levels of depression at Time 2. In other words, women who entered shelter with high levels of support from friends exhibited lower levels of depression at their time of discharge. The measure used to determine social support from friends was administered at Time 1, to most accurately assess the participant's social

	Mean	Standard Deviation
Agency	3.55	0.67
SSFam[a]	2.84	1.25
SSFr[b]	3.38	1.16
WRSt-T2[c]	5.33	1.74
WRRe-T2[d]	3.63	1.89
DepressT2[e]	2.15	0.65

Table 1: a Social support from family. b Social support from friends. c Time 2 willingness to rely on shelter staff for support. d Time 2 willingness to rely on shelter residents for support. e Time 2 depression.

Correlations among the study variables

	Agency	SSFam[a]	SSFr[b]	WRSt-T2[c]	WRRe-T2[d]	Depress T2[e]
Agency	–	.32*	.39**	.22	.08	-.18
SSFam[a]	56	–	.38**	.45*	.36	.18
SSFr[b]	56	–	.41*	.33	-.46*	–
WRSt-T2[c]	22	21	21	–	.53*	-.38^
WRRe-T2[d]	22	21	21	22	–	-.25
Depress T2[e]	21	56	56	22	22	–

Table 2: Pearson correlation coefficients are present above the diagonal; N is present below the diagonal.
^p <.10, *p <.05, **p <.01, two tailed; a. Social support from family; b. Social support from friends; c. Time 2 willingness to rely on shelter staff for support; d. Time 2 willingness to rely on shelter residents for support; e. Time 2 depression.

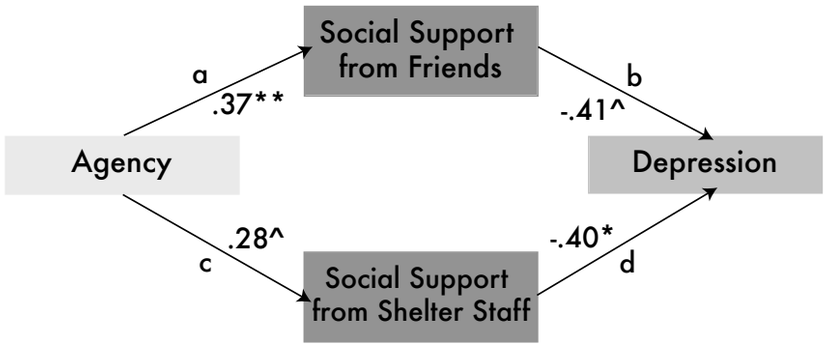

Figure 1: The standardized coefficients derived from multiple regressions are presented for ease of comparison. The coefficients for paths b and d represent the coefficients after controlling for agency and Time 1 depression in the mediational analyses. ^p <.10, *p <.05, **p <.01, two tailed.

support system before their arrival to shelter. Furthermore, social support from friends at Time 1 predicted less depression at Time 2 even when controlling for depression at Time 1. These results are consistent with previous research indicating that social support is associated with increased psychological well-being.[23-25] Results from this sample expand upon previous findings from normative samples, indicating that social support is an important element in protecting the mental health of women who have been in abusive relationships. Since many abusers tend to isolate their partners[29] and thereby limit their social support resources, this would clearly be an important area for counselors to focus on in working with women.

It is interesting that support from friends emerged as an important factor, both in terms of practical and emotional support. It is possible that relationships with friends foster connections with the greater community. It would make sense that women who are in the process of leaving an abusive relationship and establishing their own independence would need to rely on friends for practical support such as financial assistance and transportation, as well as for having another person to reach out to for emotional support. It is also important to note that while social support from family was also associated with less depression, it was not measured at statistically significant levels. This might be connected to previous studies that assert that support from family can be perceived as interference as opposed to a helpful resource.[44] Future work could usefully examine this issue further in a larger sample, to determine more definitively the relationship between depression and social support from family and friends in survivors of abuse.

The second goal of the study was to examine the relationship between experiences of support in the shelter and depression levels at discharge. Results indicated that willingness to rely on shelter staff was significantly related to lowered depression at Time 2, even when controlling for Time 1 depression. Measures of willingness to rely on support from shelter staff were taken at Time 2, in order to give the women a chance to acclimate to their new environment and gain access to socially supportive resources. This strength lends itself to the longitudinal setup of this study. These results are consistent with previous findings that social support, particularly from sympathetic and understanding people, is a protective factor against distress.[27] It is important to note that this relationship was not significant in regards to the women's willingness to rely on support from other residents in shelter. These results emphasize the impact of training shelter staff as well as the importance of effective communication between shelter staff and residents. This indicates that shelter programming, which strengthens the relationships between residents and staff, as opposed to networking among residents, would better benefit women receiving services. Furthermore, this indicates that services such as individual counseling may be more effective than group counseling sessions for reducing the symptoms of depression during a stay in shelter. The implications of these findings on applied shelter programming could be a meaningful area of future research.

The third goal of the study was to examine the hypothesis that women with higher levels of agency would report greater social support from both people outside the shelter and inside the shelter, and that this support would result in lower levels of depression upon leaving shelter. Results showed that women who exhibited agentic qualities at Time 1 also reported higher levels of social support from friends, as well as a greater willingness to rely on shelter staff, which in turn was significantly associated with lower levels of depression at Time 2. Agency, marked by an orientation toward the self such as self-direction

and self-assertion,[38] has been positively associated with social support in previous research with normative samples.[37] This study confirmed these results, indicating that agency and social support are positively associated in a sample of female residents of a domestic violence shelter. Previous studies also indicate that agency is associated with help-seeking behavior.[38] Results from this study were consistent with these findings, showing that agency was positively associated with willingness to rely on shelter staff. Furthermore, previous studies have also found that agency is positively correlated with psychological well-being and lowered levels of depression.[39] Results from this study concurred with previous findings, as women entering shelter with high levels of agency also reported decreased levels of depression at the time of discharge, even when accounting for levels of depressive symptoms during intake. This significant relationship between agency and depression can have important implications for a woman's experience in shelter. It would be effective for shelter programs to teach agentic skills, such as assertiveness, in order to help women gain the social support they need, and in turn to decrease depressive symptoms. Additionally, it is also important for counselors to keep in mind that women who come to shelter low in agency may have difficulty asking for assistance from shelter staff. These women, in particular, would benefit greatly from training in agentic skills.

Limitations

There are several important limitations to address as a part of this study. First, all data gathered from participants was in the form of self-report measures. As a result, we are unable to compare participants' responses to those of an objective observer. This increases the possibility for bias. Another important limitation to address is the reduction in sample size from Time 1 (n=58) to Time 2 (n=22). While it is generally difficult to ensure Time 2 participation in many studies, a main reason for this drop in participation is the transient nature of this particular sample. The shelter's maximum length of stay is 30-days; many women do not stay for this maximum allowance. While some women return to their abusers, and others move on to independent living, many residents desire to leave shelter and move in with friends or family; the shelter is often used a temporary safe haven at times when the woman is in fear of imminent abuse. With women residing in shelter so temporarily, it is difficult to ensure participation in Time 2 of the study.

Conclusion

Despite these limitations, the current study provides valuable information in the realm of domestic violence and experiences of abused women. Findings from this study regarding the relationship between agency, social support, and depression point to the need for continued research in this area.

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Summer 2004 REU Chemistry Scholars

Undergraduate Research Programs at the University of Rochester

A selection of programs with comments by recent participants.

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The Frederick Douglass Project

The Frederick Douglass project, sponsored by the Frederick Douglass Institute and the Department of Rare Books and Special Collections, seeks to digitize all of the Frederick Douglass materials held in the collections of the University of Rochester Library. The work will be undertaken by undergraduates, that they may have a greater understanding of Douglass by working with the letters and newspapers he composed.

Frederick Douglass spent 25 crucial years of abolitionist activism in Rochester. The University's collections hold over 100 letters that date from before the Civil War when Douglass was editor of North Star, an anti-slavery newspaper published in Rochester, to a few years prior to his death in 1895. In addition, the collection also includes photographs of Douglass and copies of his newspapers.

Each Frederick Douglass project participant is asked to transcribe letters, of their choice, written by Douglass and to use these letters in writing an essay on a topic of their choice. For more information, and previous transcriptions, essays and images of the actual letters, visit the Frederick Douglass Project website <http://www.lib.rochester.edu/rbk/douglass/home.stm> or contact Melissa Mead at mmead@library.rochester.edu.

Will Fassett:

"For my project, I examined the financial ledger of Frederick Douglass's newspaper, the North Star. I came upon this subject while reading through Douglass's correspondence in the Department's collection. A few of the letters concern the finances of the paper, and quite interestingly, there appeared to be a controversy regarding the paper's economics. In one letter, Douglass refers to a demand made by Amy Post, a close friend and fellow abolitionist, that he relinquish the financial control of the paper because of mismanagement. Wishing to investigate further what Post was referring to, I located the North Star's account book in the Library of Congress's collection of Douglass's papers. In this ledger, I found what I believed to be some irregularities in the accounting. For my essay, I described Douglass's accounting errors and discussed in detail the paper's financial failings.

"This was perhaps the best course I've taken at UR. Interacting one-on-one with Professor Jarvis and Melissa Mead was invaluable. They taught me how historians approach their subjects (namely, with questions, and more questions, and even more questions). Furthermore, I learned the joy of pursuing a subject in depth and making historical discoveries. This project has motivated me to continue my study of history at the graduate level.

"I would recommend that all students take the opportunity to work alongside a professor, as I did, and investigate a subject that they are passionate about. When you investigate something from start to finish that is entirely of your choosing, motivated by personal initiative, it is a wonderfully rewarding experience."

2002-2003 Project Interns:

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Katharine Beecher Brodock
"Antislavery, Abolitionism, and the Republican Party of the 1800s"
Advisor: Dr. Larry Hudson

Will Fassett
"The North Star, 1847-1849: Based on Evidence Found in the Paper's Ledger"
Advisor: Dr. Michael Jarvis

Eric Horan
"Commentary on Letter 83"
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Contact: Mrs. Marguerite Weston; Weston@chem.rochester.edu
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Contact: Dr. Priscilla Auchincloss; psa@pas.rochester.edu

Ben Gilston:

"I worked in Dr. Pat Holland's lab during the summer of 2004 on the synthesis and characterization of new nickel complexes using a β-dikiminate ligand

"Looking back on the REU research I did this summer I have nothing but positive things to say. The work I did this summer gave me the ability to learn the techniques I need for my senior research work, but at a slower and more manageable pace than what would have been possible during the academic year. Through the program, I was able to meet many new chemistry majors and graduate students who shared my interests. Getting to know the chemistry professors and graduate students on a more personal level was very helpful when I started the graduate school application process.

"Joining the REU program gave me the opportunity to focus a good portion of my summer on my own personal research project. Working in Dr. Holland's lab increased my desire to do future laboratory work, and helped me develop responsibility and confidence. If you are even remotely interested in doing research someday, I would highly recommend applying to the REU program and pursuing your interests."

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Laura B. Fornarola
"Studies Towards the Synthesis of Camphor-Derived Lactam Auxiliaries"
Mentor: Joseph Pero
Advisor: Robert Boeckman

Benjamin A. Gilston
"Synthesis and Characterization of New Nickel (I) Complexes using a β-dikiminate Ligand"
Mentor: Nathan A. Eckert
Advisor: Patrick L. Holland

Olesya Haze
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Advisor: William Jones

Susan L. Zultanski
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Mentor: Patrick Caruana
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Picture References

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