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The UK Undergraduate Research Program is intended to offer students, particularly in their first and second years, the opportunity to begin to engage in research and scholarship with a faculty mentor. Students in this program may enroll in a special research methods course designed to provide them with practical research and scholarship knowledge, such as how and where to seek funding, how grants are administered, using library and Internet resources effectively for research, and writing research and scholarly abstracts and reports. The following abstracts were the final papers submitted by students who took this methods course in the Spring of 2004 and reported on their on-going research.

The Neural Effects of CO₂ in *Drosophila* Larvae

Nicolas Badre, Research Assistant to Dr. Robin L. Cooper

**Introduction and Background**

Carbon dioxide (CO₂) is commonly used as an anesthetic for adult *Drosophila melanogaster*; however, the mechanism of its actions is unknown. This mechanism is important because it could possibly lead to the discovery of new types of insecticides with the potential to be innocuous to plants and plant eaters. Because mosquitoes have been shown to have sensory structures that detect CO₂, we postulated that *Drosophila* must also contain similar types of receptors, because they share the same kind of environment. Larval insects have never been examined for CO₂ sensory neurons. Previous experiments supposed that carbon dioxide affected larvae in the same way that it affects humans: an increase in body fluid acidity causing different behaviors, including anesthesia (Biston and Sillans, 1979). Those experiments also showed that CO₂ had different effects than hypoxia, because a high concentration of CO₂ and oxygen could also cause anesthesia (Sillians et al., 1969). However, the objective of this current research is to find sensory neurons on the larvae capable of detecting the CO₂.

**Methodology**

We tested Canton S, the common “wild-type” laboratory strain of *Drosophila melanogaster*. This experiment focused on larvae at the beginning of the “wandering” phase of the third instar. Many of the techniques used in this experiment were already used by Cooper and Neckameyer (1999). Each larva was in a sealed agar plate with CO₂ injected into the container. We worked in two phases.

**Phase 1 – Proving the presence of receptors**

*Body wall movements (bwm) & Heart Beats (HB)*

In phase 1, we injected CO₂ into the sealed container for a period of 10 minutes, after which the container was opened. We recorded the bwm for the first and last two minutes. If at any time bwm or the HB stopped, the time would be recorded. If the HB stopped, the time when the HB started again, once the container was open, would be recorded. The objective of this test was to quantify the difference between CO₂ and hypoxia in the larvae, using common features of the animal.

**The reaction of the larvae to the CO₂**

In our effort to identify particular characteristics of the larval response to CO₂, we coined several terms to quantify those responses. *Shell position* designates larvae that are in a curved position. *Elongated position* designates larvae that are flaccid and look longer than usual. *Contracted position* designates larvae that have returned to their normal shape after being in elongated position. The responses were tested by placing the larvae under anesthesia for approximately 5 minutes and recording the different behaviors of the larvae during the first minutes and the minutes following the end of the CO₂ injection. The objective of this test was to understand and detail the reaction of the larvae to CO₂.

We repeated the experiment with N₂ to make sure that the results were specific to CO₂. We also had a control, recording the natural bwm and HB of the larvae without the injection of any gas.

**Phase 2 – Finding the receptors**

The same flies and methods were used to take care of the larvae at this level, but sealed plates were no longer used. The larvae were placed on tape so that their movements were limited, and a needle was used to aim the flow of the CO₂. The CO₂ was projected at high pressure in order to prevent rapid diffusion, to enable the analysis of a particular section of the animal. We repeated the experiment with N₂ to make
sure that the results were specific to CO$_2$. For this experiment, the time at which the heart beat stopped was not recorded, because the time to set up the experiment was rather long and adjustments were sometimes necessary. The animal was divided into two targeted regions: the head and the tail (with the spiracles). The needle was placed accordingly without touching the larva. The aimed flow test was performed on five larvae for each gas.

**Results**

We have shown that larval *Drosophila* respond rapidly to CO$_2$ (<1 min) by freezing their body movements and contracting the spiracles (respiratory structures). Larvae exposure to 100% N$_2$ gas results in a gradual slowing down of body movement over a longer period of time as compared to the CO$_2$, and does not produce a closing off of the spiracles. Thus, we propose that CO$_2$ receptors drive the central nervous system to initiate particular motor commands that are different than those induced by hypoxia.

We have also shown that larval *Drosophila* only respond to CO$_2$ when it is projected toward the tail region of animal, suggesting that the receptors are located in the tail region.

**Future Objectives**

Our main future objective is to expand the understanding of the mechanism involved in the neural response to CO$_2$. In order to do so, we will perform a series of neurophysiologic experiments to test the response of each nerve and receptor to CO$_2$. I am currently learning neurophysiologic setups that were recently created to allow the dissection of the larvae without cutting the respiratory structures.

**Works Cited**


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**Adapting Liquid Cooling Garments for High Altitude – Low Temperature Thermal Regulation**

**Bram Bell**

**Mentor: Dr. Bruce Walcott**

Although major technological breakthroughs have allowed humans to routinely summit Earth’s highest peaks, frostbite is still a common problem that has no easy solution. My research has focused on investigating the possibility of adapting liquid cooling garments, or LCGs, to redistribute the thermal energy a climber produces while making a summit attempt. Although this research centers on a very narrow application of current technology, there are practical implications not only for mountaineers, but anyone who exerts himself or herself in extremely cold environments.

While making a summit attempt, the amount of body heat given off by mountaineers varies greatly. At higher altitudes, the temperature can drop below -50°F and the wind chill can exceed -100°F. During periods of low activity or rest, body temperature drops considerably due to the reduction in thermal energy produced. As the body cools, blood is pulled from the extremities to the core in an attempt to save the vital organs. This results in the extremities experiencing a dramatic loss of thermal energy. In most environments, when one resumes vigorous activity, the body reverses this effect and the extremities regain their lost energy quickly. In the low pressure, low oxygen environment at altitude, the body is less efficient than at lower elevations. This inefficiency extends the time necessary for the body to generate sufficient thermal energy to warm the entire body. With each cooling, the extremities may fail to regain all of the thermal energy that was lost, causing the tissue to grow colder each time a mountaineer rests.

What is needed is a way for some of the excess thermal energy produced during exertion to be stored. It can then be redirected to the extremities when activity levels drop and the extremities cool. My research stems from the idea that a liquid cooled
garment, or LCG, could be used in reverse to store thermal energy and deliver it to the extremities, thus lowering the likelihood of frostbite. An LCG is a suit that has a series of tubes attached through which a cooled liquid flows. Designed by NASA for astronauts to stay cool in their spacesuits, the technology has seen many commercial applications in diverse areas, such as firefighting, mining, and auto racing. Although much research has been carried out focusing on the ability of these garments to cool, little has been directed to heating the body or redistributing the bodies’ own thermal energy. Removing the cooling equipment reduces the system to the suit and pump, creating a unit light enough to be suitable for mountaineering applications.

There are several problems that this research will have to address. Does the LCG absorb enough energy from the core to transfer to the extremities? Is there sufficient transfer of that thermal energy to the extremities? Will too much energy be captured and induce overheating? Can the pump and battery be integrated into one unit that can be stored under the insulation layer? Can the suit fit ergonomically under the insulation layer worn by mountaineers, while still allowing freedom of movement and the wearing of a loaded backpack?

The next stage of research will address these issues and extend the practical applications of this technology. The ergonomics will be tested simply by carrying out the motions used in technical rock and ice climbing. The thermal transfer capacities of the suit will have to be tested in a cold environment such as a freezer or “cold soak chamber,” while taking measurements of skin temperatures from various points on the body.

Future research includes utilizing a more useful design for people who are not mountaineering. Freed from the constraints of weight and size concerns, practical applications of this technology are possible. Utilizing a backpack design that includes a heater and more battery power, would expand human freedom in cold environments. Current research on LCGs includes increasing efficiency by mimicking the human circulatory system and creating an actual fabric woven of microtubules.

Genotyping of Choline Transporter Knockout Mice

Tabatha Doyle
Mentor: Dr. Subbu Apparsundaram

After decades of research, the identity of the Choline Transporter has finally become clear. Choline is a vital amine that either can be a methyl group donor or used to synthesize structural membrane phospholipids and signaling phospholipids. In the lab, we are interested in Choline because it is used to synthesize the neurotransmitter Acetylcholine.

Choline enters cholinergic nerve cells through Choline Transporters (CHT) on the pre-synaptic terminal membrane. Once inside the nerve cell, it is combined with Acetyl CoA to produce Acetylcholine. If Acetylcholine is present in the body, it contributes to autonomic functions, motor activity, attention and memory, aggression, pain perception, sleep and wakefulness, temperature regulation, and thirst and feeding. If Acetylcholine is deficient it could result in Alzheimer’s disease and Schizophrenia; when Acetylcholine is in excess, it can cause Parkinson’s disease.

To further understand the role of CHT in cholinergic function and dysfunction, Dr. Randy Blakely at Vanderbilt University generated CHT knockout mice. My project during spring, 2004, in Dr. Subbu Apparsundaram’s Lab involved genotyping mice for the presence of CHT. Genotyping is required to identify the animals and correlate the levels of CHT expression with phenotypes including animal learning, choline transport levels in the brain, and acetylcholine levels in the brain. With regard to CHT knockout mice, initial studies revealed that the choline uptake in brain regions of heterozygous CHT knockout mice is comparable to normal mice, despite expressing only 50% of CHT. This finding suggests that heterozygous CHT mice have successfully compensated for the loss of CHT. We are investigating the mechanism of compensation with the ultimate aim of understanding mechanisms underlying CHT regulation. Currently, we are using CHT knockout mice for investigating the effects of estrogen and nicotine.

METHODOLOGY

All animal protocols were carried out in accordance with the University of Kentucky Institutional Animal Committee policies. C57BL/6 Mice containing one allele of CHT (heterozygous CHT knockout mice) were mated. Following the delivery, 1 cm tail snips were collected from the 14-20 day old pups. The tail snips were then used to prepare Genomic DNA. This is a 4-step process that involves cell lysis, RNase treatment, DNA precipitation, and DNA hydration. Next, the samples are used to carry out a Polymerase Chain Reaction (PCR) to amplify the DNA. After completing the PCR, a DNA Agarose Gel Electrophoresis is run at 80 hertz for one hour. The charged particles cause the DNA to migrate down the gel, forming bands at 841 base pairs (bp) and 401 bp. These bands are next captured on a UV transilluminescence image. If a band appears only at 841 bp, the mouse is genotyped as homozygous wild-type. If bands appear at both 841 bp and 401 bp, the mouse is genotyped as a heterozygous CHT knockout mouse. If a band appears only at 401 bp, the mouse is genotyped as CHT-null mouse.

RESULTS

Throughout my four month research period, I screened several DNA preparations. I found that in each screening one will identify some mice to be wild type, heterozygous, or null. The DNA from a single mouse is...
allowed to migrate in a single narrow band called a “lane.” Some lanes did not have enough DNA for PCR to take place, resulting in an empty lane. This result can be seen in lanes 4, 5, and 8 in the representative gel from one experiment at the right.

**FUTURE OBJECTIVES**

After genotyping, pups are weaned and housed in the animal facility. When the pups reach about 8-10 weeks old, these adult mice are used in different experiments that investigate the role of CHT in controlling cholinergic neurotransmission in brain regions. These experiments are designed to help understand cellular signaling pathways involved in the control of CHT function and expression. Results from these studies are expected to provide insights into mechanisms involved in the control of cholinergic neurotransmission in physiological and pathophysiological states.

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**In vivo examination of hydroxyurea and the latest ribonucleotide reductase inhibitors trimidox and didox in combination with doxorubicin: suppression of uncontrolled, abnormal cell growth due to breast carcinoma**

By: Amanda M. Fleming  
Mentor: Dr. Vincent S. Gallicchio

**Background**

Inhibition of ribonucleotide reductase (RR) is a newly developed strategy for improving breast cancer chemotherapy. The need for advances in chemotherapy has gained attention, due to the development of severe cardiac toxicity induced by doxorubicin, an anthracycline used in chemotherapy treatment for breast cancer. Doxorubicin eradicates cancer cells by binding to DNA and stimulating DNA damage. Cytoxan (Cyclophosphamide) is an alkylating agent also used in breast cancer regimens that interferes with DNA synthesis and repair. It cross-links with the nucleotides of DNA, further preventing the DNA double helix from unwinding. This study supports the idea of using ribonucleotide reductase inhibitors (RRI) in combination with Doxorubicin (Dox) or Cytoxan (Cyt) to enhance the survival of breast cancer patients.

The RR inhibitors Trimidox (TX) and Didox (DX) are the primary focus of this study. Hydroxyurea (HU) is another RR inhibitor used in this study, but it differs slightly from the other two. Studies with HU encouraged development of DX, which is a hydroxyl-substituted benzohydroxamic acid derivative of HU. TX’s and DX’s abilities to produce antitumor effects are due to their function as a chemotherapeutic target. They inhibit the enzyme that catalyzes the reduction of ribonucleotides into deoxyribonucleotides, the precursors of the deoxynucleotide tri-phosphates (dNTP) used in DNA synthesis and repair. Alterations of dNTP supplies cause DNA fragmentation and cell death by apoptosis, which is an ideal mechanism for eliminating cancer cells and does not cause damage to surrounding tissues. Additionally, RR inhibitors act as antioxidants, enabling stabilization of the damaging free radicals produced by Dox and, therefore, reducing toxic effects to the heart.

HU differs from TX and DX in that it acts primarily as an iron chelator rather than an antioxidant. HU bonds to iron molecules, which are necessary for free radical formation. However, studies have shown DX to inhibit the enzymatic activity of ribonucleotide reductase 17 times more effectively than HU. The diagrams below show the primary chemicals in our studies.

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**Methodology**

Mice were inoculated with mammary tumor cells. Beginning two days after inoculation, the mice were treated with Dox, Cyt, DX, TX, and HU alone or in combination. Animals were monitored daily and tumor measurements were taken three days a week. Body weights were recorded on a weekly basis. Upon completion of the study, the effects of RRI + Dox and RRI + Cyt were determined by plotting survival rates against treatment type. All drug treated groups showed enhanced survival versus tumor controls. All Cyt + Dx were alive and showing no signs of tumor after 60 days. Cyt mono-therapy animals survived 52 days. Dox + Dx survived slightly longer than Dox mono-therapy animals. DX mono-therapy animals survived 21-26 days. From experimental results, it is logical to conclude that combinations of DX + Dox allow animals
to survive longer than Dox mono-therapy, and that DX enhances the anti-tumor effects of Cyt.

Conclusions
In vivo assessment of RRIs has revealed prospective treatment strategies for breast cancer. Future studies will assess the need for alternative therapies for HIV infection. RR plays a role in the HIV virus and may aid in inhibiting HIV replication, also.

References


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The Effects of Temperature on Neuromuscular Development in Larval Drosophila
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Introduction
Different and changeable environments lead to biological adaptations for proper functions. In spite of the available information about temperature effects on the Drosophila, the mechanism in the functional relationships of temperature effects over various developmental stages has not been provided in detail. It is known however that higher temperatures (25-30°C) cause larval Drosophila to grow more rapidly as compared to colder temperatures (15-18°C). As the animal develops, so must the skeletal muscles. By examining the development of the neuromuscular junction (NMJ) in larval Drosophila grown at different temperatures, some of the basic processes in the development and maintenance of the synaptic transmission might be revealed. Morphological differences of the nerve terminals can be examined at different levels. The objective of this research is to provide pertinent information regarding the effect of temperature on development of larval Drosophila with particular attention to the development of the larvae neuromuscular junction.

Methodology
As soon as Drosophila eggs were hatched, the animals were placed into one of the following temperature environments: 18°C, 25°C, or 33°C. The larvae full body length, abdominal longitudinal muscle 6 (m6) in segment 4, and outgrowth of the neuromuscular junction changes were used as morphological indices for the whole animal and motor unit development.

Results
When comparing the different growth rates for each temperature, the 1st instars did not have significant differences in body length. However, at 18°C, longer developmental periods were present for the 2nd instars and 3rd instars. The relative cumulative frequency from the mean values indicated that there is a faster rate of development for the higher temperature. Also the organisms showed a decrease in the locomotor activities at lower temperatures. With use of HRP staining, preliminary results indicate that the larva raised at 33°C developed more branched nerve terminals. It was serendipitously found that the HRP antibody can be used on a preparation even after the tissue has been processed with mounting media. This also opens up the possibility of using secondary antibody staining, if the need arises in future studies.

Conclusions
The results suggested a tight correlation between the temperature and development rate in the body level. There was a difference in the varicosities and branches of the NMJs, which supported the different temperatures affecting the nerve terminal morphology. From this study, we concluded that different temperatures lead to dramatic difference in larvae development. This conclusion leads to further investigations at the anatomical and the physiological level to understand the NMJ performance at different temperatures.

Future Studies
Measurements for comparing the muscle dimensions and the NMJ development are still in progress in or-
Homeless Men in Temporary Settings with Families

Shanna Sanders, Research Assistant to Dr. Joanna M. Badagliacco, Department of Sociology

I am assisting Dr. Joanna Badagliacco in a study that focuses on homeless families in rural Kentucky, and the physical or sexual abuse that male household heads have encountered throughout their lifetime.

Dr. Badagliacco has interviewed 102 women in rural Kentucky homeless shelters about their viewpoints, childhoods, relationships, past and present sexual and physical abuse, experiences with substance abuse and alcoholism, income level, education, and children. I am examining the 16 interviews conducted with the men married to these women in the study. Before this time, the only homeless families that had been interviewed consisted of women and their children. It is rare, as seen from these interviews, for the male member of the household to stay with his family after becoming homeless.

Dr. Badagliacco has written a number of papers concerning homeless families’ experiences with violence, the intergenerational transmission of poverty, and inequality. She teaches and researches social inequalities, especially with respect to families in poverty.

My responsibility has been to input quantitative data from the men’s interviews into the SPSS (Statistical Package for the Social Sciences) database that contains 400 variables from the interviews. After this data set was created, statistical information, such as means and frequencies, were calculated from the data I input. I also examined the qualitative information that respondents supplied. It was then possible for me to see correlations among the interviewed men.

A voice is given to the homeless families in rural Kentucky through this research project and Dr. Badagliacco’s upcoming manuscript. The homeless and the causes behind this country’s homelessness must be understood before we can begin to solve the problems associated with this situation.

During this research project, we discovered that these men had experienced overwhelming amounts of both physical and sexual abuse throughout their childhoods. As an example of the physical abuse these men have encountered in their past, 80% of the men interviewed responded “sometimes” or “frequently” when asked if, “Before the age of 17, an adult threw something at, pushed, grabbed, shoved or slapped you.”

We also discovered that these men had little education, most were not high school graduates. Although all had jobs, the average income for these men was less than $10,000 annually. Because of their past experiences, these men could not be sufficient providers for their wives and children.

These men are faced with a daunting task. They have become fathers, a role for which they had no adequate model to learn from. Their childhoods were spent in unstable households, moving around, being faced with physical and sexual abuse, and being exposed to substance abuse and alcoholism. They do not know how to become the fathers they need to be.

Unfortunately, if something drastic does not occur, these men are likely to only pass the cycle of poverty on to their children. Change is necessary before homelessness in families becomes an even greater problem in the United States. We have proposed that public policy be altered so that the homeless can learn the skills necessary to live on their own.

Many future research possibilities are available related to these topics. At some time, I would like to examine statistical differences on these same questions between the homeless men with families and homeless men without families, homeless men who have left their families, and housed men with families.
Uranium Immobilization with Borax

Railey White, Research Assistant to Dr. David Atwood

Uranium has been used in this country and around the world for many decades, but its use poses a serious threat to our environment. It has applications ranging from supplying heat to our homes to supplying the most destructive weapons the world has ever seen. But, regardless of its application, the use of uranium has the same unpleasant side-effects in the form of waste. One Department of Energy (DOE) facility that is attempting to manage such waste is the Gaseous Diffusion Plant in Paducah, KY. Currently, the uranium contaminated water in Paducah is contained in large holding tanks waiting for a method of remediation. These large tanks of UF₆ (uranium hexafluoride) could produce large amounts of aqueous Uranyl (\([\text{UO}_2]^{2+}\)) which is the basis of our study (Friedman, 2004).

Brandon Conley (a former UK undergraduate researcher who is now on an NSF graduate student fellowship at M.I.T.) proposed that because the uranium is in an ionic form we should be able to find a counter ion, equal in size and charge, which would bind with the uranyl to form an insoluble precipitate (Conley, 2003). Conley and Atwood were funded for this study by the Kentucky Science and Engineering Foundation (KSEF) (Grant: 12-217-RDE-002 “Actinide Immobilization in Groundwater”). Several counter ions were tested, but they did not cause uranyl precipitation; however, promising results were obtained with selected boron reagents.

In our studies, we used an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) to determine the amount of uranium in solution. This instrument passes the sample through a plasma torch and captures the wavelength(s) of light emitted; the intensity of the wavelength is used to calculate the concentration of the sample. Each of the studies we conducted utilized this instrument. First, we conducted a concentration dependent study that held the uranium concentration constant and varied the concentration of the boron additives. The samples were centrifuged and the supernatant was decanted and analyzed. From this concentration dependent study, we determined the optimal concentration of boron for the best uranyl removal. This is now the basis of a UK patent application.

“Use of Boron Compounds to Precipitate Uranium from Water.” After the concentration dependent study, we wanted to determine whether or not the precipitate formed would leach back into solution with time. The utility of our remediation technology would be reduced if the boron-uranium precipitate proved to be unstable. For the leaching study, eight sets of identical samples were created at ratios of 20 times and 30 times the amount of uranium present, and within each sample set, pH 4, 6, 8, and 10 were tested. These samples were centrifuged and left for 1, 7, 14, or 21 days, then analyzed. The results from this study showed that within this four week period, there was no significant difference in the amount of uranium in solution from day 1 to day 21. This test also confirmed our results from the previous study, that the higher amounts of boron remediates best, and that pH = 4 is the optimal pH level for removal.

A corollary study to the leaching procedure was conducted, which analyzed the pellet formed from the precipitate after centrifuging. This procedure was used to determine when precipitation occurs, and to measure the concentration of uranium in the pellet. One sample set was analyzed on days 1, 7, 14 and 21. On each day the samples were analyzed, centrifuged, the supernatant decanted, and the pellet was re-suspended into 1% Nitric Acid solution. This re-suspended pellet was analyzed, and it was determined that the vast majority of precipitation occurred before day 7.

Another study was conducted to determine the effect of surfactant (sodium dodecyl sulfate) on removal. The addition of surfactant to the samples resulted in no increase of uranium removal.

Our characterization of the precipitate indicates that it is a new material. A sample of the precipitate was sent to Borax, Inc. for characterization, and their results confirm our findings.

Based on these results, further characterization will be conducted as well as possible testing at increased concentrations of boron. Also, in the studies we conducted, centrifugation was used as a means to simulate gravity over time; however the real length of time required for achieving similar results is yet to be determined. Further investigation of these and other variables will help us to understand how boron compounds bind to the uranyl ion, and could help us achieve commercial application of this process.
