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My Experience Exploring the Effects of Lead (Pb +2) Toxicity in Drosophila melanogaster using Sociability Interaction Testing and Microarrays at the University of Puerto Rico-Rio Piedras in San Juan, Puerto Rico

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"My Experience Exploring the Effects of Lead (Pb +2) Toxicity in *Drosophila melanogaster* using Sociability Interaction Testing and Microarrays at the University of Puerto Rico-Rio Piedras in San Juan, Puerto Rico"

written by

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Backstory

It started with a hot day in August. I was excited to start my new work-study position in the Biology department. After all, I loved the outdoors, and how many other positions in the work-study program offered such a luxury? My first task began the day before classes started. Dr. Knight entrusted me with the department's pickup truck, and I headed to Hardman's Hardware to load up several 45 pound bags of soil to replenish the supply in the greenhouse.

Wanting to prove myself as a competent and trustworthy employee, I decided to unload the bags that same day. I bent my knees, and carefully, with my back as straight as a toothpick, scooted one bag of soil onto each shoulder off the tailgate. I lifted with my legs and satisfactorily walked to the large storage container inside the greenhouse. Twenty minutes passed, and after an unrecalled number of trips the truckload was empty. It was the next morning when I woke up with the pain. It felt like a knife stuck out of my right scapula. The pain would persist for over a year undiagnosed.

As the semester continued daily activities began to take more effort and energy. I had been to several doctors, and before things were over would see eight of them about my particular situation. Several things loomed in my mind, one of the prominent ones being how I would conduct successful research with my injury. It was that time of my college career, and without it I could forget graduating with a BS.

Each week Dr. Knight would send an e-mail or two about off-campus opportunities. Many of the e-mails I received about research contained adventurous opportunities all over the world; the only problem was they were only volunteer—no pay. Eventually an e-mail advertising bioinformatics summer research through INBRE (IDeA Network of Biomedical Research Excellence) arrived. I had no idea what bioinformatics actually was. The pay was an

estimated $4,000. It also offered research at various institutions, and I could sign up for any of them as long as it was not my home state. I signed up with the University of Puerto Rico in Rio Piedras as my preference.

Eventually the time came for students to apply for a summer research position with one of the professors at OBU. Still not having heard back from INBRE, I signed up to avoid the likelihood of doing research during the business of the semester in order to meet the requirements. A couple of weeks passed and I learned I had been chosen to work with Dr. Taylor on his plant research. This worked out well in my mind, because if I was still sick by the time summer rolled around, at least home would not be too far away if I needed some kind of therapy or treatment to get better.

Around mid-semester, with everything set to go with Dr. Taylor, I heard back from INBRE. It was surprising at first to learn that I had been accepted into the INBRE program. It was a tough decision to decide between working with Dr. Taylor and traveling to Puerto Rico. In the end I felt it was the right thing to allow someone who needed to meet their research requirements work with Dr. Taylor. However, I was also worried about living in Puerto Rico for two months while trying to function through my chronic pain, but God made things possible.

Upon arriving at the airport in San Juan in May my new summer boss, Dr. Humberto Ortiz-Zuazaga, was there to pick me up. Our conversation began when he asked if I was hungry. I said yeah, and that some rice and beans would hit the spot. Coincidentally, he mentioned that at his house he and his wife were leading a student Bible study and had a home cooked meal. I had been on the island less than a day and had already found a place to attend church.

At the Bible study that night I was introduced to some of the other students doing research at the Univeristy of Puerto Rico in Rio Piedras. The room was diverse with a

Panamanian, Haitian, Peruvian, Russian, several Puerto Ricans, and an American present. The windows were open to let in the night breeze, and outside I could hear the Puerto Rican frogs known as *coquís* sing their song. The moment was special.

I traveled with Humberto the next day to pick up my roommate from the airport. He was a short Vietnamese student named Ai Tran from Tulane University in New Orleans, LA. Living in a suite together, we tended to get along fairly well because we each had our own room. Our first few days of work consisted of a programming language course, when my boss realized that neither Ai nor I had any background in computer programming, unfortunately.

Eventually Ai went to work with Dr. Steve Massey, a British professor of Bioinformatics who had also given us a tour of Old San Juan earlier that week. With my background in Biology, Humberto sent me to work with his wife, Dr. Sandra Peña-Ortiz, who was collecting the data Humberto was using for his graphs by extracting RNA from fruit fly brains. So, the week after my programming crash course began I was learning to extract brains out of fruit fly larvae. Originally I was going to learn how to do RNA extraction the first day in Dr. Sandra's lab. However, while cleaning out the minus 80°C freezer, one of her grad students threw away a week's worth of brains. I was the fix-up crew, and spent that week and the weekend listening to Pandora and pulling out brains. It was actually quite relaxing.

In general, as one walks down the streets of San Juan, he does not pay attention to, or succumb to any of the numerous beggars that approach him. There is a certain degree of street smarts, shrewdness, and common sense necessary to avoid the con-artists. Ai and I lived off of Walgreens. It was right down the road, and very often for lunch or supper we ate canned vegetables along with *pinchos*, which are pieces of chicken, pork, or steak grilled on skewers

sold by street venders. One night as I waited for Ai outside of Walgreens one such homeless man approached me.

As he paced around in front of me talking about how bad his life was, I politely listened nonetheless. He had a daughter in Silicon Valley who left him and never sends him money, he did not do drugs, the Priest at the church would not offer him any help, and neither George Bush nor Barack Obama would give him any money. He must have assumed to have fooled me when he proceeded to ask for my money so he could purchase the cigarettes I did not have. Instead I invited Jorge, the man's name, to grab some *pinchos*. Eventually Ai came out of the store, and I could tell he had no idea how to take the situation, so he rolled with it.

As we ate our meal along the street Jorge talked loudly with a big goofy grin, all the while espousing his wisdom and view of the world. He may have been crazy, but I was amazed how he could be homeless and know so much. I believe he had had a college education. I noticed one of his homeless buddies grinning mischievously as he watched his friend conn two suckers into buying him food. To me all that mattered at that moment was to leave my mark in Puerto Rico. Being so sick while there, I never went to the beach or jungle, and never even left San Juan, but I wanted to be sure that the hands and feet of Jesus had moved through me on that island before I left.

While doing my best to live as a witness in Ai's life, so was another person, my boss's wife. Sandra had had a difficult time in recent years. She had overcome cancer, but it left her with what I could tell was some sort of neuropathy. After having attended their church for some time, I was learning more and more about Sandra and her family. Her youngest son, Isaac, was autistic. Through Sandra I saw love and science intertwine as each day in the lab she researched to learn more and more about her son's illness. On top of taking care of her family, Sandra was

the President of INBRE in Puerto Rico. She would casually call Ai and I to check on us. Although she rarely came to the lab because of her condition, she always seemed on top of things and would occasionally ask us if we were learning new things.

Humberto and Sandra also came to pick me up and some of the other students at UPR each Sunday morning for church. They belonged to a church group known as the Local Church movement. Each Sunday began with the congregation seated in a circle around a table, on top of which lay bread and grape juice. After songs were sung out of a hymnal, Bible study books were passed around until each congregate had one.

The main difference I noticed in the room was the absence of a pastor or preacher. Instead of a sermon, members of the congregation would discuss the interpretation and personal significance of the Bible studies inside the books that were passed around. One Sunday after church I searched the name written on the study book, Witness Lee, on the internet. I found several controversial articles debating the Christian versus cultic nature of the Local Church movement.

The Local Church movement arrived in America in 1962, when it was brought over by Witness Lee, a student of the movement's founder, Watchman Nee. One of the principle beliefs of the Local Church movement is in the unity of the church as opposed to the existence of denominations. However, as can be seen in the following quote from Witness Lee, the wrong idea can be communicated. "We do not care for Christianity, we do not care for Christendom, we do not care for the Roman Catholic Church, and we do not care for all the denominations, because in the Bible it says that the great Babylon is fallen. This is a declaration. Christianity is fallen, Christendom is fallen, Catholicism is fallen, and all the denominations are fallen.

Hallelujah!" Many have blamed quotes such as these on Witness Lee's bad English and failure to assimilate his teachings into a Western context (Myer et al., 2007).

As I continued to attend this church and study it, I did always appreciate the oneness of the congregation and the ability of the brethren to value each of its members by offering open participation. On Sundays I never noticed any behavior among the congregation that made me uncomfortable or feel that it was strongly unbiblical. However, I believe the absence of a pastor made it difficult for the congregation to learn and grasp some of the denser theological teachings of the Bible.

After all of the research I did on the Local Church, I decided to continue attending Sunday service with them. I think there is a lot of misunderstanding on both sides between the Local Church movement and other denominations. The brethren in Puerto Rico did not discriminate against me when I told them I attended a Baptist church, and I think they liked the fact that there was interdenominational fellowship between us. I could see the Holy Spirit moving in many of their lives upon getting to know them better.

My work in the lab was progressing, but unfortunately there were also a lot of setbacks. I spent most of the time working with Adrinel, Sandra's employee. She was a sweet lady, and even took me out to lunch a couple of times whenever she was around in the lab. Because we did not have the right equipment with which to disrupt the proteins in our RNA extraction process, Adrinel attempted to use a makeshift method, and I believe for the most part that it denatured the proteins on several attempts.

Between denatured proteins and a deadline, I spent most of the summer extracting brains. Eventually I was able to extract one brain every five minutes, which was quite difficult considering the hours spent hunched over the microscope and the small size of the tweezers. It

was also an improvement compared to the embarrassing two brains I extracted after an entire day of work in the lab on the first day. Occasionally one of the other students who were working in the same lab, but on a different project, would come by my station and ask if they could try to extract a brain. The Puerto Rican students in the lab and I got along well, and we all enjoyed listening to *reggaeton,* Puerto Rican-style hip hop, while we worked at our different stations.

Near the last couple of weeks of the eight week program I was sent to work in the "fear room," which was where Sandra kept all her mice for fear conditioning and her study on the amygdala of the brain. However, also in the "fear room" was where the fruit fly Sociability Interaction Testing (SIT) took place. There I reviewed hours of recorded data of flies walking back and forth in plastic tubes, typing in ones and zeroes in an Excel spreadsheet every time a fly crossed the interaction zone. Eventually I would hand this data over to Humberto in Bioinformatics, and he would translate it into a graph.

The last week in Puerto Rico was the most difficult. In the previous seven weeks on the Island sometimes my pain would be so bad I would have to call my dad late at night. That is a hard thing to do as a grown man. In fact, I called my parents every day, almost sucking the life out of them for the encouragement I could not muster on my own after dealing with the unknown pain for a year. In that last week all the residents were being cleared out of the dorm building where Ai and I lived, so we had to move to the one next to it. Before, Ai and I had had separate rooms, but now we would share one. It possibly would not have been a big deal normally, but now I had no privacy where I could more or less sober up from a painful day. During that last week it was hard to stay positive about my relationship with Ai. With the way things were, it was discouraging to feel that I had not made any impact upon his life. At least not that I could tell at the moment. I would not hear from him again until a year later in an e-mail.

On one of the last nights on the Island I was invited to dinner at the house of one of the church members. There was one moment at the table where I could not use my right arm to reach for something because it would have hurt. Sandra used the moment to illustrate how we all help each other out with our strengths where others are weak. She said we were all one family and body. It was a simple illustration, but it meant a lot to me after feeling so alienated from the world because no one, not even the doctors understood my injury.

If I had to describe the Puerto Rican people in one word, it would be "hospitable." During the two months on *la Isla del Encanto*, an affectionate name for Puerto Rico meaning "Island of Enchantment," perhaps one of the largest factors that kept me persevering was the love of the friends I had made. Naturally, doing research in Puerto Rico is a dream. There are beautiful beaches, nice weather, friendly people, and delicious food. However, sometimes a beautiful country-side bike ride can be a chore if you have a loud squeaky wheel. It is moments like those in life when you feel lonely and hurt, and you find yourself before the face of God. You can even see God weep.

Fortunately, after returning home from Puerto Rico I was finally diagnosed with some herniated discs in my thoracic spine and was able to undergo therapy so they could heal. I wonder sometimes how it would have been to be in Puerto Rico that summer uninjured. How many more people would I have been able to meet and influence? What sort of amazing sites and places would I have been able to travel to? In the end, I trust that all things work for the good of those who believe. Below contains a content concerning our research findings.

Effects of Lead (Pb^{2+}) Toxicity in Drosophila melanogaster using Sociability Interaction Testing

Abstract

Lead is an environmental contaminant widely dispersed throughout the world. Exposure to lead causes neurological damage in humans and may be linked to neurodevelopmental pathologies such as attention deficit hyperactivity disorder, antisocial behavior, and autistic spectrum disorders (ASD). *Drosophila melanogaster* (fruit flies) have been used to understand the behavioral, synaptic, and molecular changes that occur after developmental exposure to lead and to study ASD-associated pathology. Flies were exposed through the mother and until eclosion to either a lead-laced medium or a control corn-based medium and were isolated until the beginning of the behavioral studies. Sociability testing was done using a test tube that was divided by a mesh allowing the flies to interact using olfactory and visual clues, while avoiding direct contact. Multiple fly recordings were made using a high-throughput video system and analyzed using "Freeze Frame" software from Coulbourn Instruments. Our findings indicate that developmental exposure to lead results in dose-dependent developmental delay and decreased social interaction in female flies.

Introduction

With more than 80,000 commercial chemicals in use today, and some 2,000 new chemicals introduced each year, it is important to study and learn the neurological effects of these chemicals upon humans and other organisms (Rand et al., 2010). It is also important to know the effects of heavy metals and other naturally occurring toxins commonly present in the environment (Rand et al., 2010). For 4,000 years the toxicity of lead (Pb^{2+}) has been known to man. With the use of leaded gasoline and paint in the 20^{th} century the amount of the heavy metal

in the environmental increased drastically (Hirsch et al., 2010). The fruit fly, *Drosophila melanogaster*, serves as an excellent model for this research for reasons discussed below. Therefore, this paper will in part discuss the practicality and benefit lying behind the use of *D. melanogaster* for studying the effects of lead toxicity on living organisms. After discussing the reason for the use of *D. melanogaster*, the symptoms and plausible causes for Autism Spectrum Disorder (ASD) in humans will be discussed. I will then review the sociability interaction testing experiment and how it relates to what may happen with humans exposed to lead in their developmental years.

Why the Fruit Fly?

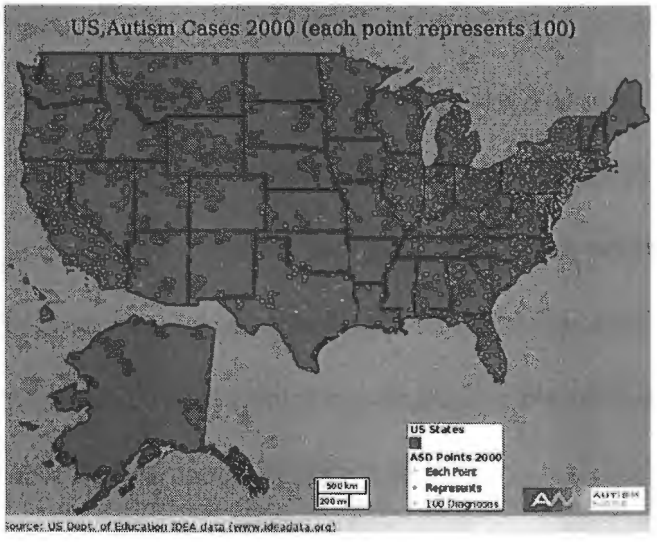
D. melanogaster has been used in mutagenicity tests for several reasons (Rand et al., 2010). One is the low cost to maintain the organism. For example, in a lab setting *D. melanogaster* is typically fed cornmeal in 20-50 mL vials. It also has a short life cycle. Five days after fertilization the female adult will lay the eggs. In approximately 24 hours the embryo develops and a fully functional nervous system with sensory and motor neurons forms (Rand et al., 2010). At 25°C it will require less than a day for the eggs to hatch into larvae. The larvae will then mature through the first, second, and third instar stages in approximately four to five days (Atkins et al., 1992). During the transition from larva to pupa, the fly will molt twice, and grow ten times in size (Rand et al., 2010).

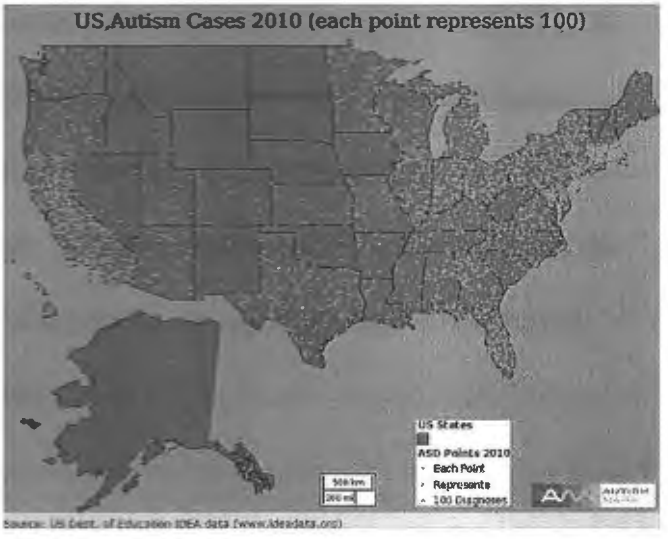
Once a pupa, *D. melanogaster* only lacks four to five more days before becoming an adult (Rand et al., 2010). Thus, within roughly ten days a whole new generation of fruit flies are ready for experimentation. Fruit flies are useful for neurological experimentation because of their genetic manipulability and easily interpreted phenotypes (Rand et al., 2010). Their size, quick maturation, and affordability facilitate testing a large number of organisms at once (Atkins

et al., 1992). Because so many specimens can be tested when using *D. melanogaster*, more confidence can be placed in the data.

Autism in Humans Today

In the past two or three decades the number of autism diagnoses has increased immensely. In California, where records of cases have been kept systematically, cases have increased by 634% from 1987 to 2002. (Liu et al., 2010). Below are two geographical maps of the United States showing the numbers of Autism Spectrum Disorder (ASD) cases in 2000 and 2010.





While the number of autism diagnoses is increasing, it is uncertain whether autism itself is becoming more prevalent in children, diagnostic methods are improving, children are being over-diagnosed with autism, or a combination of the three. To make things more enigmatic, risks traditionally associated with an increase in autism prevalence are now being questioned by research. The plausible causes for autism include environmental toxicants, genetic predisposition, prenatal conditions, obstetric complications, and parental characteristics. This paper will primarily cover autism as related to environmental toxicants, and it will lightly touch

on genetic predisposition. Other variables that have been considered in studies are race, premature birth, socioeconomic status, occupation, and history of schizophrenia (Liu et al., 2010).

Public opinion and changes is societal perceptions of autism has also been shown to effect the number of diagnoses. In one case it is shown that middle to upper-class families, opposed to having their child diagnosed as mildly or severely retarded, prefer to have them diagnosed with autism (Liu et al., 2010). Thus, it does seem that the rise in documented cases of autism cannot solely base itself on an increase of the disorder. According to two separate studies, it is estimated that between 25% to 33% of autism diagnoses are simply a change in what would have traditionally been diagnosed as mental retardation (Bishop et al., 2008; King et al., 2009). It is also believed that before the jump in cases of autism, it may have been underdiagnosed due to its unpopularity as a publically perceived psychiatric disorder, as opposed to a medical one (Liu et al., 2010). The increase in diagnosed cases of autism, of course, is not wholly based on changes in diagnostic methods either.

Autism as Caused by Heavy Metals

As of 2010, 272 toxicants have been linked as probable contributors to autism (Liu et al., 2010). In 2005 in Kuwait a conducted a study on trace elements present in the hair of children and their connection to autism (Fido et al., 2005). Kuwait serves as an excellent study environment due to the radical modernization that has occurred in the past 20 years within the nation spurred on by the growth of the oil industry and the Gulf War. Of those involved in the study, children with autism were shown to have higher levels of uranium, lead, and mercury in their hair when compared to the healthy-controlled group of children (Fido et al., 2005). The mean levels of lead in blood in the United States has significantly decreased since graduated

disuse of leaded gasoline and paint. However, even in areas considered environmentally free of lead contamination, the heavy metal can still reside in the bones of individuals years later. These deposits of lead are then later released from the bones during pregnancy or lactation, and can be passed to the vulnerable fetus or baby (Hirsch et al., 2009).

Autism as Caused by Genetic Predisposition

But not only can the risk of autism increase from environmental contaminants, autism is also considered the most genetic neuropsychiatric disease by the scientific community (Liu et al., 2010). The basis for a genetic explanation in increased cases of autism lies in the fact that siblings are more likely to exhibit the symptoms. However, only 15% of autism cases have been shown to have a known genetic cause, and only 1-2% of this 15% is due to only one cause (Liu et al., 2010).

Currently the most plausible answer for the rise in autism cases that has recently been recorded is an increase in the age of parents at the time of birth. In 1992 the percent of the population born to parents over the age of 35 was 24.3%. Then, in 2000 the population of children born to parents over 35 had increased by 36.2% (Liu et al., 2010). The increase in the likelihood a child may have autism if born to older parents is thought to be principally due to *de novo* mutations (Liu et al., 2010).

De novo mutations are changes to genes not seen in the genetic make-up of the parent, but rather in their sperm or egg. Therefore, whereas a parent may not be autistic, he or she can transfer the condition depending on his or her age and the extent to which mutations have accumulated in his or her gametes. This in itself may not alone cause autism. Sometimes *de novo* mutations may exist, yet genes responsible for autistic behavior are not expressed to a noticeable extent until environmental toxicants or some other factor encourages an unwanted

increase in expression. Suppression of genes responsible for normal behavior combined with environmental toxicants could equally result in the same outcome. The problem is simply that the mechanism between genetic expression and heavy metal toxicants is poorly understood.

Developmental Lead (Pb^{2+}) Exposure in D. melanogaster

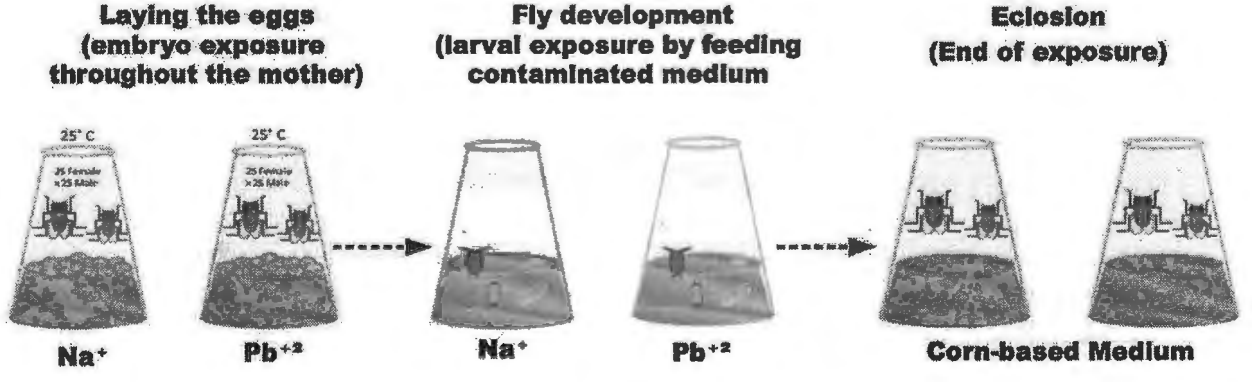
When *D. melanogaster* are exposed to lead during the developmental stages of their life cycle, they show signs of slowed development (Hirsch et al., 2003). This deviation from proper maturation does not only occur at one stage of development, but throughout the process (Cohn et al., 1992). The size of the fly is not affected, which therefore indicates the cause of the retardation is not of nutritional origin (Hirsch et al., 2003). It is the neuromuscular junctions and behaviors whose development depends on neuroplasticity that are primarily affected (Hirsch et al., 2003). The experience dependent growth of the nervous system assembly is altered (Morley et al., 2003). As a result, flies exposed to lead in developmental stages are shown to respond to certain actions more quickly than normal, whereas a normal fly would delay in responding to those actions. An example of this would be a courtship ritual, where the increase in mating impulsiveness in fruit flies indicates that the females are not selectively searching for a mate, but are rather readily interacting with their first male encounter (Hirsch et al., 2003).

It is also found that prolonged exposure to lead during development influences activity-dependent synaptic plasticity in the mammalian brain (He et al., 2009). In general, *D. melanogaster* response to lead results in similar symptoms as those seen in humans, and therefore it likely serves as an excellent model to study the relation between lead toxicity and ASD and its signs and symptoms (Hirsch et al., 2003). In order to study the neurological effects caused by chemicals and toxins, such as lead in this case, it is necessary to first identify the phenotypes expressed by organisms exposed to the heavy metal. From there the genes that play

a role in neurological function and development can be further studied. Common symptoms and signs exhibited by those diagnosed with ASD can include poor social skills, abnormal nonverbal communication, intense repetitive behaviors, decreased sensitivity to pain, increased sensitivity to sound, uncoordinated fine motor skills, and coordinated gross motor skills.

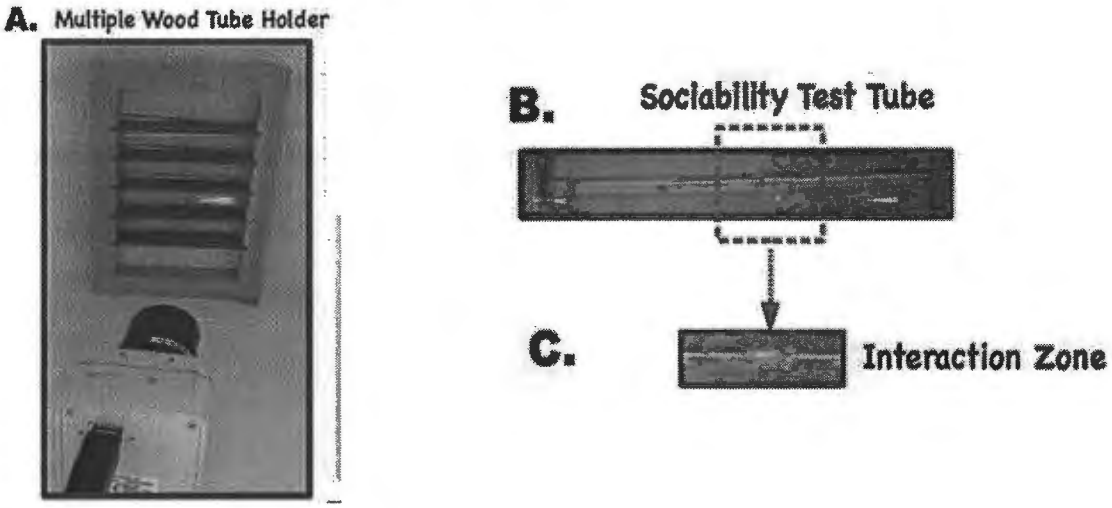
Materials and Methods for Sociability and Interaction Testing

For the sociability and interaction testing (SIT) experiment, wild type Canon S female fruit flies were either crossed in standard cornmeal media in the presence of 500 μM sodium (Na^{+}) or in the presence of 500 μM lead (Pb^{+2}) Acetate at 25°C. After the flies had mated and the female eggs had been laid, the fruit flies were removed from the media container as the cycle of the new laid eggs began. One group of flies was exposed to the sodium or lead media across development, the other was exposed only to sodium or lead media in the adult stage. Those exposed during development were transferred after eclosion to standard (non sodium or lead) corn media for three days before SIT. Adults who were not exposed to lead or sodium during developmental stages were placed, after eclosion, in sodium or lead contaminated media for 24 hours and then transferred to standard corn media more two more days before SIT. A depiction of the flies being exposed through development is seen in the figure below.



Again, this process takes approximately 12-14 days (Rand et al., 2010). At the time of their eclosion the newly hatched flies are used for Sociability and Interaction Testing (SIT).

In SIT two tubes of 4.4mm in diameter by 40mm long each and joined by an elastic chamber of the same diameter were placed together as seen below in figure B.



A fly was placed in each tube, with one on each side of the elastic chamber, which can be seen depicted in figure C. The flies were not permitted to pass through the chamber due to a cloth mesh in the middle. A lead-exposed fly was placed in one side of the tube, while a control sodium exposed fly was placed in the other. The mesh allowed the flies to interact using their visual and olfactory system, while avoiding direct contact. The test tubes were placed in a custom-made "multiple wood tube holder" (8 in by 8 in), which held up to six pairs of test tubes at a single time and is depicted above in figure A. The pairs of test tubs were separated by a compressed wood panel so that the flies on the different levels of the "wood tube holder" did not see each other.

After a 30 minute habituation period had passed, recordings were made using an automated video tracking system, which consisted of a video camera connected to Freeze Frame software for automatic counting made by Coulbourn Instruments. Each recording lasted for six minutes. Social interaction was recorded by focusing in on the interaction zone, as seen in figure C above. The time each fly spent in the interaction zone was analyzed, but only counted as interaction if both flies were in the zone while simultaneously facing one another. Each time the flies were officially interacting, a "1" was placed at that time interval (seconds). Below in figure D, each peak on the scale represents a moment when the flies were recorded present in the interaction zone.

D. Freeze Frame Software for automatic counting (Coulbourn Instruments)

The recorded video was then analyzed, and each time a peak was reached the video was played in slow motion so that the viewer can note if the flies ~~and~~ are facing one another and interacting, or simply the interaction zone at the same time per chance. The data was then sent to Dr. Humberto Ortiz-Zuazaga who used bioinformatical techniques to create graphs.

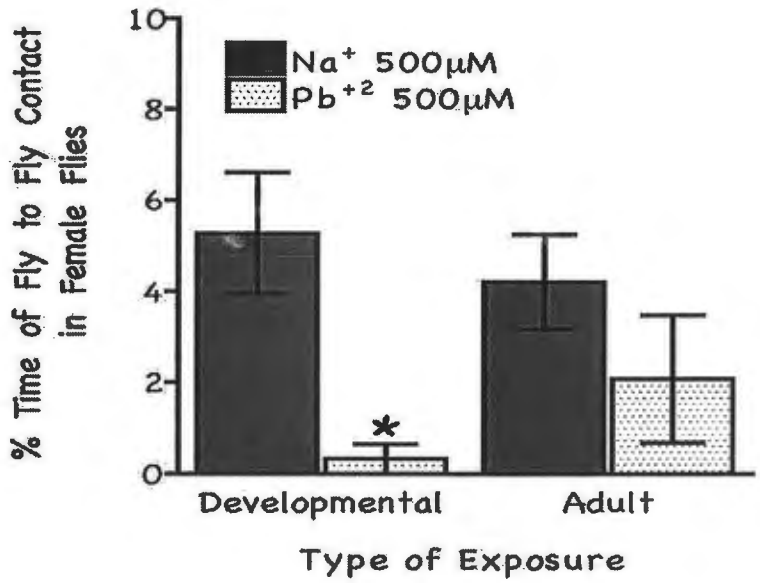
Materials and Methods for D. melanogaster Brain Extraction

Wild type Canton S third instar larvae were used for the brain extraction process. In the same manner in which flies were reared for SIT, some flies were raised in lead based media, while others were placed in the control sodium based media. RNAsezap was used to eliminate RNAses from the working environment. RNAases are enzymes that catalyze the breaking down of RNA. They are present on our skin, and therefore it was also necessary to wear gloves during the extraction process. The working station therefore cleaned by RNAsezap prior to each session of brain extraction. Once the larvae reached its third instar life-stage it was placed on a petri dish, which was placed under a microscope. Using fine tweezers, the *D. melanogaster* larva was pulled apart, with one pair of tweezers grasping at the head, and other pair at the opposite end. Once this was done, excess material was removed until the upper half of the body was left.

The tweezers were then used to pull at the ventral nerve, which is the major nerve running down the body of the larva starting at the brain. If done right, the brain came out of the upper portion of the body. After this was completed, excess material around the brain is removed until a clear two-lobed object lies in the petri dish. The two-lobed object, the brain, is then placed in a tube with RNA-later, which will prevent the denaturation of the RNA so that the brain tissue may be later used in another project for RNA extraction and genetic microarray testing.

Results

The graph below shows a significant decrease in the percent of time of fly to fly contact for those flies exposed to lead during their developmental cycle.



*P=0.0094 P=0.7899

The low P-value for developmentally exposed fruit flies indicates that the chance of this result randomly occurring is low, and therefore it is very likely there is a link between the developmental exposure and the decrease fly to fly contact. The higher P-value for the adult exposure indicates that the lower contact time for flies exposed to lead is not of great significance.

For fruit fly brain extraction I developed a new technique using tweezers. Typically special fine-tipped scissors are used for the process, but because our lab lacked the equipment it was necessary to find a new and efficient method. I later taught this method to other employees in the lab and to Adrinel Vázquez-Montes, the lab technician.

Discussion and Conclusion

Drosophila melanogaster serves a suitable model for studying toxicants in the environment and their effects on developing organisms. With the discrepancy between the

percent of time spent in fly to fly contact between developmental lead exposed and developmental sodium exposed it can be concluded that lead does in fact effect the neuroplasticity through experience behaviors. The lack of evidence for a significant effect of lead toxicity upon adult *D. melanogaster* further indicates that changes in neuroplasticity is the primary cause for the deficit in social behavior seen in the flies. In the future further studies may be conducted to identify the gene regulatory networks associated with lead mediated sociability impairments in the adult fly.

Bibliography

Atkins J.M., Schroeder JA, Brower DL, Aposhian HV. 1992 Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine.

Bishop DV, Whitehouse AJ, Watt HJ, Line EA. "Autism and Diagnostic Substitution: Evidence From a Study of Adults With a History of Developmental Language Disorder" Developmental Medicine and Child Neurology. 2008;50:341–45.

Cohn J, Widzowski DV, Coy-Slechta DA. 1992 Lead retards development of *Drosophila melanogaster*. Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology. Vol 102 (1) pages 45-49.

Fido A., Al-Saad S. 2005. Toxic Trace Elements in the Hair of Children with Autism. In Sage publications and the National Autistic Society

He T, Hirsch HVB, Ruden DM, Lnenicka GA. 2009. Chronic lead exposure alters presynaptic calcium regulation and synaptic facilitation in *Drosophila* larvae. In Neurotoxicology pages 777-784.

Hirsch HVB, Mercer J, Sambaziotis H, Huber M, Stark, DT, Torno-Morley T, Hollocher K, Ghiradella H, Ruden, DM. 2003 Behavioral Effects of Chronic Exposure to Low Levels of Lead in *Drosophila melanogaster*. In Neurotoxicology pages 435-442.

Hirsch HVB, Possidente D, Averill S, Despain TP, Buytkins J, Thomas V, Goebel WP, Shipp Hilts A, Wilson D, Hollocher K, Possidente B, Lnenicka G, Ruden DM. 2009. Variations at a Quanititaive Trait Locus (QTL) Affect Development of Behavior in Lead-Exposed *Drosophila melanogaster*. Neurotoxicology pages 305-311.

Hirsch HVB, Possidente D, Possidente B. 2010. Pb^{2+}: An Endocrine Disruptor in *Drosophila*?. Physiology and Behavior pages 254-259.

John Myer. 2007. A Future and a Hope: Church Life beyond the Local Church Movement.

King M, Bearman P. "Diagnostic Change and the Increased Prevalence of Autism" International Journal of Epidemiology. 2009;38:1224–34.

Liu K., Zerubavel N., and Bearman P. 2010. Social Demographic Change and Autism.

Morley EJ, Hirsch HVB, Hollocher K, Lnenicka GA. 2003. Effects of Chronic Lead Exposure on the Neuromuscular Junction in *Drosophila* Larvae. In Neurotoxicology pages 35-41.

Rand, MD. 2010. Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. Neurotoxical Teratol pages