Cellular Stress Response and Emergency Room Visits of Patients with Intellectual and Developmental Disabilities

A Major Qualifying Project
Submitted to the Faculty of
Worcester Polytechnic Institute

In partial fulfillment of the requirements for the
Degree of Bachelor of Science
By:

_____
Brianna Burke

Date: April 24, 2019

Approved:

_____
Professor Natalie Farny, Advisor

_____
Professor Brenton Faber, Advisor

Table of Contents

Table of Figures:

Project Description:

This Major Qualifying Project (MQP) was completed to satisfy the requirements of both the Biology & Biotechnology and Professional Writing degrees. The purpose was to expand on the cellular research done for the Biology & Biotechnology portion by conducting an epidemiological study of Emergency Department (ED) Visits for patients with Intellectual and Developmental Disabilities (IDD). These two studies worked collaboratively to provide groundwork for further studies to evaluate the health and care of individuals with IDD. To complete the combined MQP, I conducted the lab work with my team: Renee LeClaire, Lindsey Merrill, and Solimar Ramis de Ayreflor Reyes, under the guidance of Professor Natalie Farny. Simultaneously, I conducted an epidemiological study evaluating the ED visits of patients with IDD under the guidance of Professor Brenton Faber. This MQP worked as an exercise in scientific writing and working to provide breadth and depth in knowledge while maintaining integrity and understanding of correlation versus causation.

This full MQP report is a compilation of the work done for both majors. The first chapter contains the team-written report done by Renee LeClaire, Lindsey Merrill, and Solimar Ramis de Ayreflor Reyes, and myself. The second chapter contains the individually-written epidemiological report.

Acknowledgements:

Biology & Biotechnology MQP

We would like to thank Dr. Nancy Kedersha of Brigham and Women's hospital for the U2OS-DS cells, and Dr. Joel Richter of UMass Medical School for the mouse fibroblast and human lymphoblast cell lines. We would also like to thank Dr. Louis A. Roberts, Dr. Michael A. Buckholt, and the BBT department at WPI for assisting us throughout our MQP. We especially appreciate our advisor Dr. Natalie Farny for her guidance and support throughout the MQP process.

Professional Writing MQP

I would like to thank Canton-Potsdam Hospital for their assistance and for providing the data needed to complete this study. I would also like to thank Canton-Potsdam Hospital and WPI IRB for their guidance through the approval process. Finally, I thank Dr. Brenton Faber for his guidance and support through the approval and development of this MQP.

Chapter One: Investigating the Impact of Fragile X Syndrome on Stress Granule Formation

Abstract

The protein FMRP is missing in Fragile X Syndrome (FXS) patients. Stress Granules (SGs) are cytoplasmic bodies where mRNAs are stored during cellular stress to inhibit their translation. FMRP localizes to SGs and regulates mRNA translation; therefore, we hypothesized that SGs may differ in FXS and unaffected cells. We used fluorescence microscopy to quantify SG formation in wild type and FXS mouse embryonic fibroblasts and human B lymphocytes. Our results suggest altered stress responses may contribute to the pathophysiology of FXS.

Introduction

Cellular Stress Response

Cellular stress is defined as an introduction to, or a change of, a stimulus that damages the structure and function of macromolecules or the cell itself (Poljšak *et al.* 2012). These stimuli can lead to damage of proteins, DNA, other molecules, or cell death (Kultz 2005). To combat these stresses, cells have evolved numerous different mechanisms to tackle stressors of various levels called cellular stress response (CSR). One of the mechanisms is the formation of stress granules. These are created so the cell can survive until the stressor subsides.

Stress Granules

A common method of CSR is the formation of stress granules, which have been found across yeast, protozoa, and metazoa (Anderson and Kedersha 2009). Stress granules are clusters of untranslating messenger ribonucleoproteins (mRNPs) which form from stalled mRNAs (Protter and Parker 2016). Stress granules are most often formed after translational initiation is halted due to stress-induced phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α). During the halted phase, elongating ribosomes are unharmed and simply fall off the stalled polysomes. This results in a circularized, polyadenylated mRNA transcript that is still attached to cellular pre-initiation machinery (Anderson and Kedersha 2009). Figure 1 summarizes the formation and breakdown of stress granules within a cell (Dobra et al. 2018).

Stress granules are believed to sort and degrade mRNA during times of cellular stress and recovery. They can also assist with mRNA regulation and stability (Anderson and Kedersha 2009). In addition, stress granules recruit other molecules which can affect the equilibrium of associated molecules. These molecules can also shift the cellular environment into a different stage. For example, stress granule recruitment of antiviral proteins during an infection enhances innate immune response (Protter and Parker 2016).

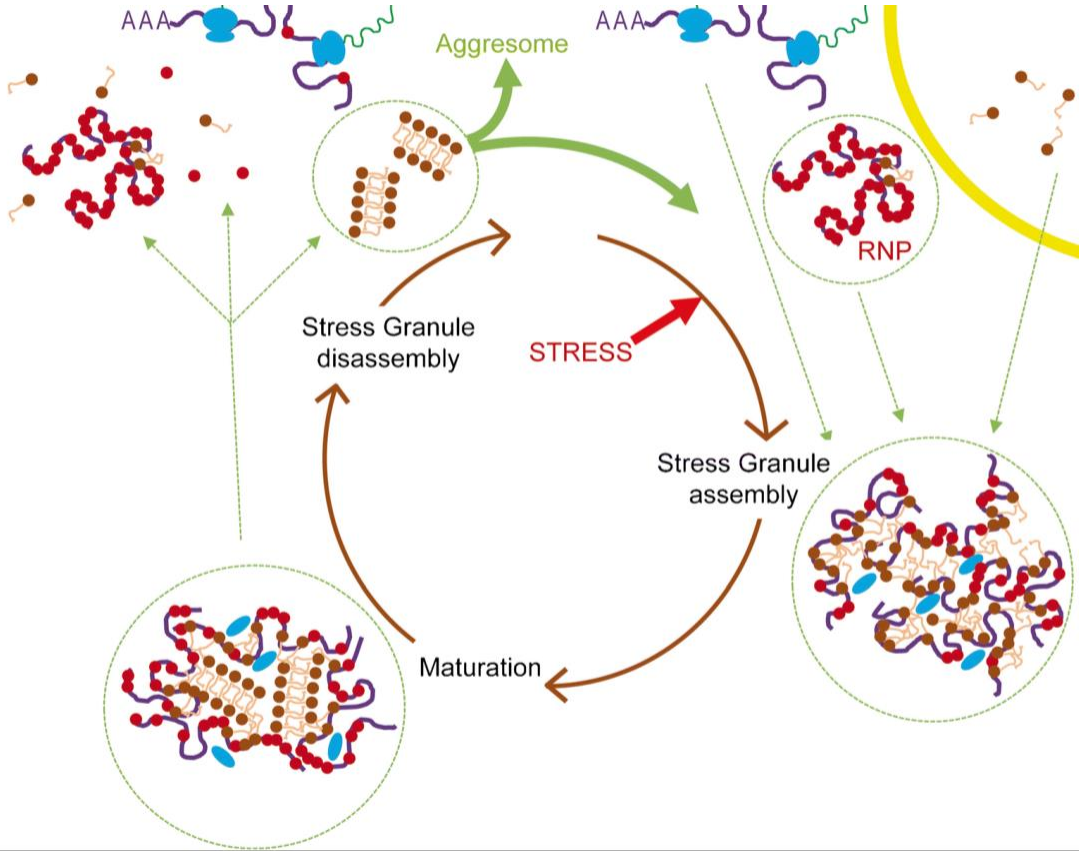

Figure 1. Assembly of Stress Granules (Dobra et al. 2018)

Figure 1 displays the formation and breakdown of stress granules in the cytoplasm. The mRNA originates in the nucleus, which is outlined in yellow, and enters the cytoplasm. RNPs bind to mRNAs, which aggregate in the stress granule so the genetic material is preserved until the stress subsides. Some proteins are also present in stress granules. Aggresomes assist in degradation of proteins once the stress granule disassembles (Dobra et al. 2018).

Bisphenols

Bisphenols are made of two phenols with bridging molecule(s). They are commonly used to manufacture plastics and polycarbonates (Konieczna *et al.* 2015). Bisphenol A (BPA), as shown in Figure 2, is a public health concern due to its function as an endocrine disrupting molecule, and its analogues are of growing concern as well. BPA is composed of two phenols with a bridging carbon that has two methyl groups attached to the carbon (Chen 2002). Steps have been taken to eliminate BPA from plastic products, but it is often replaced with its analogues, such as bisphenol S or bisphenol F. Despite this removal, BPA is a compound of emerging concern and most people in the developed world are exposed to it on a daily basis.

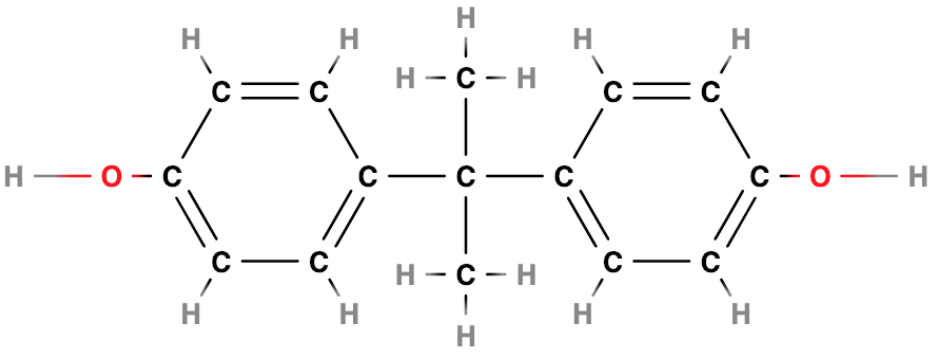

Figure 2. Bisphenol A (BPA) molecular structure

Figure 2 shows the bridging carbon between two phenols. There are also two methyl groups attached to the central carbon.

Bisphenol A (BPA)

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, also known as BPA, is an organic synthetic chemical that is produced worldwide (Konieczna *et al*. 2015). BPA was first used in 1891 and in the early 1950s it began to appear in industrial and consumer products (Vogel 2009). Since the mid-1970s, BPA has been considered a chemical with high-volume production (Vogel 2009). Over six billion pounds of BPA are produced each year worldwide and an additional 200 tons of the chemical are let out into the atmosphere during its production (Ritter 2011).

Common products that contain BPA are food containers, baby bottles, toys, water pipes, cell phones, laptops, and medical equipment. Humans are exposed to BPA in their everyday lifestyle. In fact, the 2003-2004 National Health and Nutrition Examination Survey discovered 92.6% of the participants had traces of BPA in their urine (Antonia *et al*. 2008). BPA concentrations in these urine samples ranged from 0.4 µg/L to 149 µg/L and differed between races, household income, gender, and age (Antonia *et al*. 2008). Frequent exposures to BPA can occur through inhalation, ingestion, and absorption from dermal exposure.

In 1993, BPA's endocrine disruption potential was discovered by endocrinologists at Stanford University. They were searching for an endogenous estrogen in yeast but instead came across BPA from their polycarbonate flask. The endocrinologists' research found that BPA was the chemical competing with estradiol for estrogen receptors and this was not a product of the yeast that they grew in culture. Their published results lead to more research by other scientists on the endocrine disruption potential of BPA (Krishnan 1993).

The human tolerable daily intake, which is defined by the United States Environmental Protection Agency as the maximum amount of a substance a human can be exposed to daily without adverse effects, of BPA is approximately 50 µg/kg/day. However, adverse effects have been detected at lower concentrations of BPA. Low dose effects of BPA have been linked to many diseases including birth defects, neurodevelopmental disorders, cardiovascular disease, some cancers, and autoimmune disease amongst many more (Rochester 2013). BPA activity

frequently resulted in genetic damage, epigenetic changes, endocrine disruption, oxidative stress, and/or cell signaling (Rezg *et al.* 2014).

Autism Spectrum Disorders and Fragile X Syndrome

Autism spectrum disorders (ASD) are a class of genetic disorders; ASD affect an individual's social skills, communication, and behavior (Lord *et al.* 2000). People with ASD may react differently to environmental contaminants than people without ASD. One ASD is Fragile X Syndrome (FXS), which is caused by extra CGG repeats in the fragile X mental retardation 1 (*FMR1*) gene promoter region (Hall and Berry-Kravis 2018). An individual with FXS often has varying levels of "intellectual disability, autism, seizures" (Hall and Berry-Kravis 2018). FXS affects more males than females since the *FMR1* gene is located on the X chromosome (Davidovic *et al.* 2011). Males also have more severe symptoms than females (Hall and Berry-Kravis 2018). The number of repeats present in *FMR1* determines if the individual is unaffected, a carrier, or has FXS (Hall and Berry-Kravis 2018). The CGG repeats in the promoter silence the *FMR1* gene, therefore it cannot translate the fragile X mental retardation protein (FMRP), which participates in protein synthesis at the synapse in neurons (Davidovic *et al.* 2011; Hall and Berry-Kravis 2018).

FMRP binds within the coding region of translating mRNA, which slows the elongation rate of translating ribosomes (Darnell *et. al* 2011). Therefore, FMRP represses translation. Also, there is evidence that FMRP is present in stress granules and may affect the nucleation of stress granules (Anderson and Kedersha 2008). The relationship between FMRP and stress granules is depicted in Figure 3. Since FMRP is not present in individuals with FXS, they may have increased translation and form stress granules differently than people without FXS.

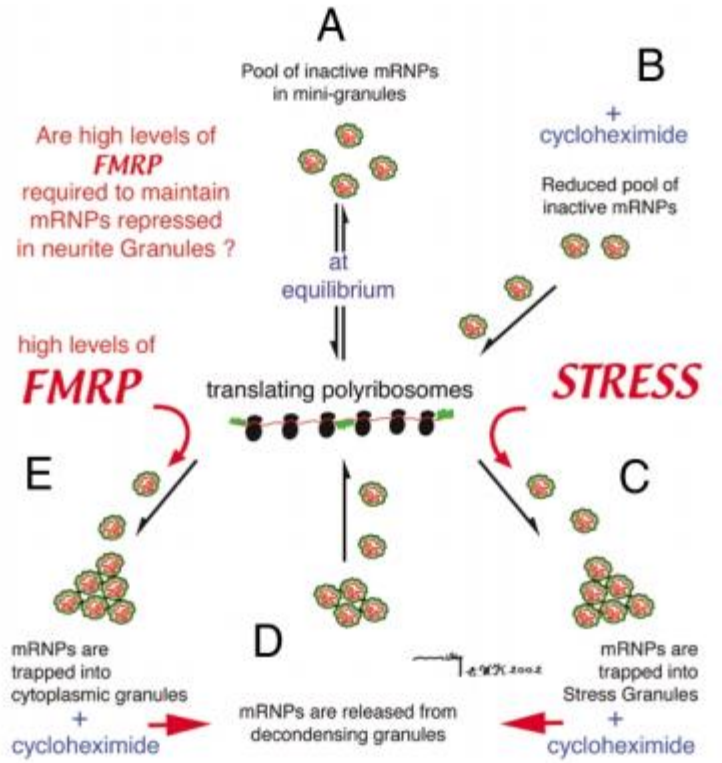

Figure 3. The role of FMRP in stress granule formation (Mazroui *et al.* 2002)

Figure 3 displays the relationship between FMRP and stress granule formation. Both FMRP and stress granules regulate translation. FMRP binds to translating mRNAs, which hinders the movement of ribosomes as they move along the mRNA. Stress granules appear to preserve mRNA during times of stress and no translation occurs in stress granules (Anderson and Kedersha 2008; Mazroui *et al.* 2002).

In addition, high levels of FMRP might control the repression of mRNPs (Mazroui *et al.* 2002). To learn more about the connection between FMRP and stress granule formation, Gareau *et al.* conducted a study to determine FMRP's effect on stress granule formation in *Drosophila* since the localization of FMRP in their stress granules is conserved (2013). The study found that FMRP is a component amongst many other proteins that are involved in stress granule nucleation. Furthermore, Gareau *et al.* found that FMRP shuttles in and out of stress granules, however, it does not alter nor is necessary in *Drosophila* cells for the formation of stress granules (2013). Therefore, cells that do not produce FMRP can still form stress granules because they have other proteins involved in the nucleation process. Still, the absence of FMRP alters the protein composition of the stress granule (Gareau *et al.* 2013) and presumably alters the mRNA profile as well. It remains unclear what effects if any these changes in stress granules may have for individuals with FXS.

FMR1 knockout mice show an increase in protein synthesis of about 20%, which contributes to the effects of FXS on the brain (Udagawa *et al.* 2013). FMRP binds coding regions of mRNA; in *FMR1* knockout animals their ribosomes translate excessive proteins from the mRNA because they can move freely (Udagawa *et al.* 2013). In order to make a stress granule, the translating ribosomes must detach or run off from the mRNA so the mRNA can become part of the stress

granule. Since ribosomes are able to move with more freedom in *FMR1* knockout cells than in wild type cells, the absence of FMRP may affect the dynamics of stress granule formation.

Experiments addressing concerns about the effects of daily plastic use on the overall population have been conducted, although few have examined the effect on smaller populations, like those individuals with FXS. BPA, a component of many plastic products, is known to cause a stress response in various cell types (Friend *et al.* 2018). In this study we examined stress granule formation in response to BPA in mouse embryonic fibroblasts (MEF) and human B lymphocytes that were either wild type or affected with FXS. We hypothesized there would be a difference in the dynamics of stress granule formation between the wild type and FXS affected cells when they were exposed to varying concentrations of BPA. Because ribosomes would run off from translating polysomes more quickly in the absence of FMRP, we predicted that mRNAs in FXS cells would be able to move into stress granules in a shorter amount of time or at lower concentrations of environmental stressors. We find that FXS patient lymphoblasts are more sensitive to stress granule formation in response to the stressor sodium arsenite, but we see no significant difference in stress granule formation in response to BPA. The results suggest that, at least in response to some stressors, that FXS-affected cells may be more sensitive to stress, which could contribute to the pathogenesis of this disorder.

Materials and Methods

Cell Line Maintenance

Wild type and FXS affected mouse embryonic fibroblasts and human B lymphocytes were a kind gift from Dr. Joel Richter (University of Massachusetts Medical School, Worcester, MA). Double stable GFP-G3BP and RFP-Dcp1 U2OS cells (Kedersha et al., 2008) were a generous gift from Dr. Nancy Kedersha (Brigham and Women's Hospital, Boston, MA). The mouse embryonic fibroblasts and double stable GFP-G3BP and RFP-Dcp1 U2OS cells were maintained in 1x DMEM (Corning Cellgro, Catalog No: 10-013-CV) with 10% fetal bovine serum (Equitech-Bio, Inc., Catalog No: 3FBU3132-0500) and 1% penicillin/streptomycin (Lonza, Catalog No: 17-602E). The human B lymphocytes were maintained in with 1x RPMI (Sigma Life Science, Catalog No: R8758),10% fetal bovine serum and, 1% penicillin/streptomycin.

Adherent cells, mouse embryonic fibroblasts and double stable GFP-G3BP and RFP-Dcp1 U2OS cells, were subcultured approximately every 2 to 3 days. Cell media was removed from the flask and discarded into a waste beaker. A Phosphate-buffered Saline (PBS) rinse was completed and also discarded into the waste beaker. 1.0 mL of Trypsin (Lonza,Catalog No: CC-5012) was added to the flask. The flask was then put in the 37°C incubator for 2-4 minutes to allow the cells time to detach from the flask wall. After the flask was removed from the incubator, the flask was rinsed with new DMEM media. The new cell containing media was split appropriately into the two new flasks; the ratio was between 1:2 and 1:8. More DMEM media was then added to each flask to bring the total volume to 14 mL.

Suspension cells, human B lymphocytes, were also subcultured every 2-3 days. Cell media was split into respective ratio and placed in new flasks. Additional mixture of 1x RPMI media with 10% fetal bovine serum and 1% penicillin/streptomycin was added into each flask to reach a total volume of 14 mL.

B Lymphocyte Cells Pre-Plating

Coverslips and ultrapure water were autoclaved. The coverslips were then placed one per well on the plates and covered with 0.5 mL of polylysine solution (Sigma Life Science , Catalog No: P4707). The polylysine coating was left on for an hour in a 37℃ incubator. After one hour incubation, the polylysine solution was removed and the coverslips were rinsed with 0.5 mL of autoclaved water three times.

Cell Plating and Pre-Treatment

The cells were plated in 12 well plates containing coverslips at concentrations ranging from 8 x 10^4 - 1.2 x 10^6 cells per mL. Depending on the cell type, 1 mL of either DMEM or RPMI based media was placed into each well before incubating the cells at 37°C for approximately 1-2 days. After incubation, 0.5 mL of media was removed from each well and combined with media that contained the same cell type and was receiving the same treatment. A 0.1M stock solution of BPA was diluted in preconditioned media to achieve the final molar concentration indicated in each experimental sample. An untreated sample, the negative control, and an arsenite treated sample, the positive control, were also prepared. Figure 4 shows an example of how the 12 well plates were utilized for plating and treatment. 0.5 mL of the newly mixed media and reagent were added back to each of the appropriate wells and the plate was incubated for one hour at 37°C.

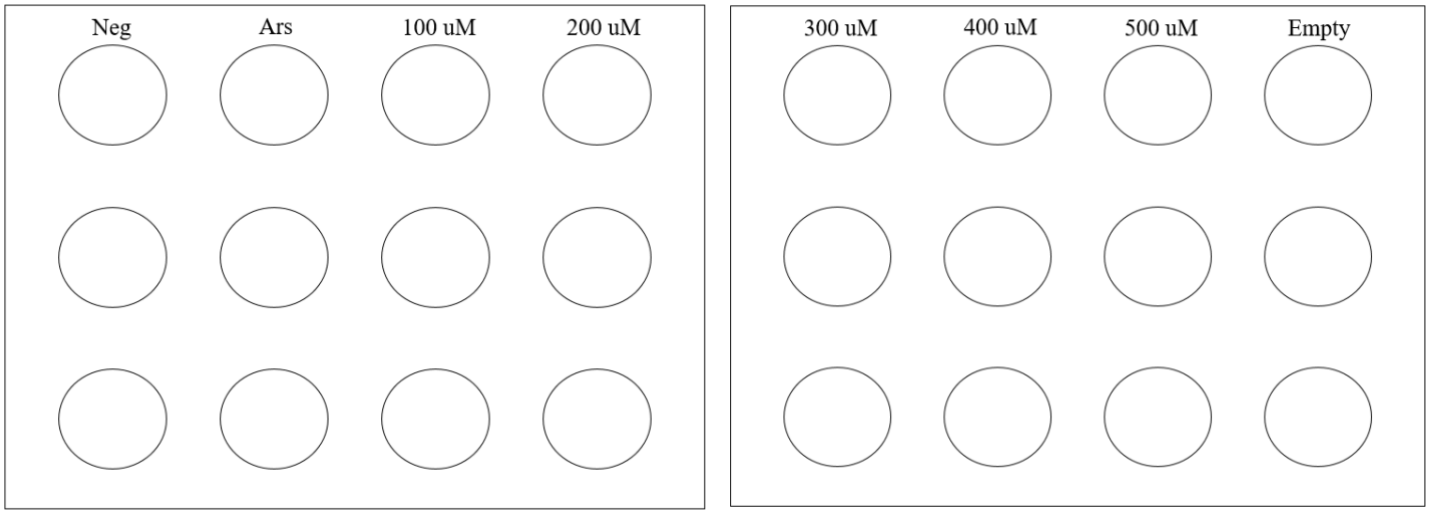

Figure 4. Acute Exposure Assay Treatments

The treatments for their respective columns are labelled. Neg is negative control, or untreated. Ars is cells treated with 100uM of arsenite. 100uM-500uM represent the concentrations of BPA added to each well. One of the columns was left empty because only 7 treatments were utilized.

Cell Fixation

Fixation was done in order to stop any changes the cells were undergoing (reacting to the treatment, dividing, etc). Once the cells were removed from the incubator, and placed in the hood, all media and reagent was removed from each of the wells and placed in the discard beaker. The wells were rinsed twice with PBS, which was removed and placed in the discard beaker. 0.5mL of 4% paraformaldehyde was placed into each well and the plate was placed onto the shaker for 10 minutes on medium. After 10 minutes, the plate was brought back into the hood and the paraformaldehyde was removed from each well and placed into the hazardous waste bottle. 0.5 mL of 1% triton detergent was put into each well and the plate was placed on the shaker for an additional 10 minutes. After the second 10 minutes on the shaker, the plate was again brought back into the hood, the detergent was removed and placed in the hazardous waste bottle. The wells were rinsed with PBS twice, the PBS was removed and placed in the hazardous waste bottle each time. PBS was then placed in each well a third time, enough to cover the cover slides. The plate was then placed in the refrigerator for storage or brought to the bench for next steps.

Antibody Staining

The cells were stained in order to see stress granules using fluorescence microscopy. 0.5mL of blocking solution, which consisted of 1% PBS and 5% normal horse serum (NHS), was added to each well being treated and the plates were left on the shaker for approximately 1 hour. The blocking solution was removed and 0.5 mL of a solution containing a ratio of 1uL of the primary mouse monoclonal (Abcam, Catalog No: #181150(EPR3986(B)) antibody anti-G3BP to 1mL of

blocking solution was added to each well being treated. The plates were placed on the shaker for approximately 1 hour. The primary antibody solution was removed and 3 1% PBS washes were conducted for 5 minutes each. The plates were put on the shaker during each wash. A solution containing a ratio of 1uL of hoechst (Life Technologies, Catalog No: 333342) to, 1uL of secondary anti-rabbit (red) antibody (Cell Signaling Technology, Catalog No: 8889S), to 1mL of 5% NHS + PBS was prepared. 0.5mL of the solution was added to each well being treated and the plates were wrapped in tinfoil and placed on the shaker for 1 hour. The solution was removed and 3 PBS washes were conducted for 5 minutes each. The plates were put on the shaker during each wash. The coverslips with treated cells were mounted with polyvinol mounting media as described (Fukui et al., 1987) after completing the antibody staining protocol.

Data Collection and Statistical Analysis

The slides were blinded using tape to cover their respective treatment concentration or control type. The slides were then analyzed using a fluorescent microscope (Zeiss, Vert.A1, AXIO). The microscope lens was set to 20X. Two to three fields were counted, to obtain a total of 250-300 cells. The percent of stressed cells was calculated by dividing the number of cells that contained stress granules by the total number of cells counted on that specific cover slip. The percent of nonstress was calculated by dividing the number of cells that did not have any stress granules by the total number of cells counted on that cover slip. Each coverslip was counted at least two times. The various counts were then averaged. Experiments were repeated three times unless otherwise indicated. Error bars represent the standard error of the mean. Differences between samples were analyzed using ANOVA and paired t-tests, as indicated in the figure legends. Statistical analyses and data collection were performed using Microsoft Excel.

Results

Verification Trials

Verification trials using U20S-DS and human B lymphocyte cell lines were conducted to ensure that the acute exposure assay would yield cells with visible stress granules and that each group member was counting cells, either with or without stress granules, accurately. The cells were treated with varying concentrations of BPA for one hour. The cells treated with arsenite served as a positive control and untreated cells served as the negative control. Cell fixation ensued treatment to allow for scoring of stress granules using a fluorescent microscope.

The first verification trial was performed using U20S-DS cells, which are known to form stress granules when exposed to arsenite and BPA (Friend *et al.* 2018**).** The data collected in the trial is shown in Figure 5**.** Raw data for the U20S-DS verification trial can be found in Appendix A.

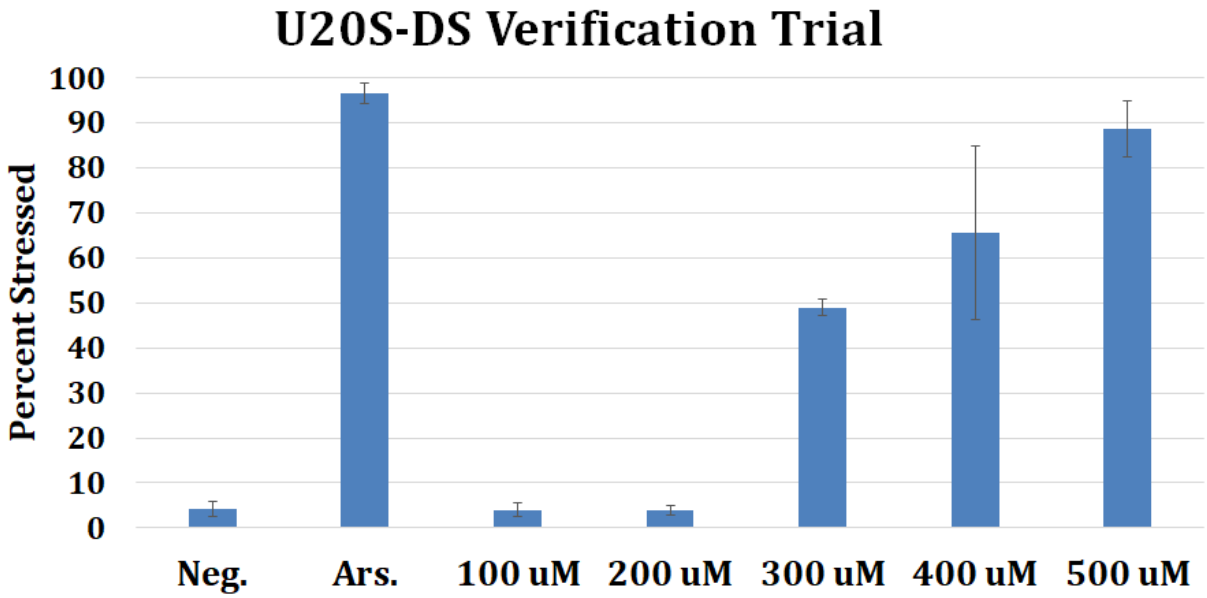

Figure 5. The average percentage of U20S-DS cells exhibiting stress. These cells were treated for one hour with varying concentrations of BPA, 500 uM of arsenite, or left untreated as indicated in the figure. One biological replicate was performed. Error bars within the figure represent standard error.

The results were consistent with previous work and showed a dose dependent response as anticipated. One biological replicate was completed and counted by two team members. Standard error was calculated and shows the variance between the two counts. These results provided confidence that the assay would work throughout the experimental period.

A second verification trial was performed using the human B lymphocytes to ensure that the wild type (unaffected) and FXS affected cell lines would produce a stress response. Again, untreated cells served as a negative control and arsenite treated cells served as a positive control. Figure 6

shows the results from the human B lymphocyte verification trial. Raw data for the human B lymphocyte trial can be found in Appendix B.

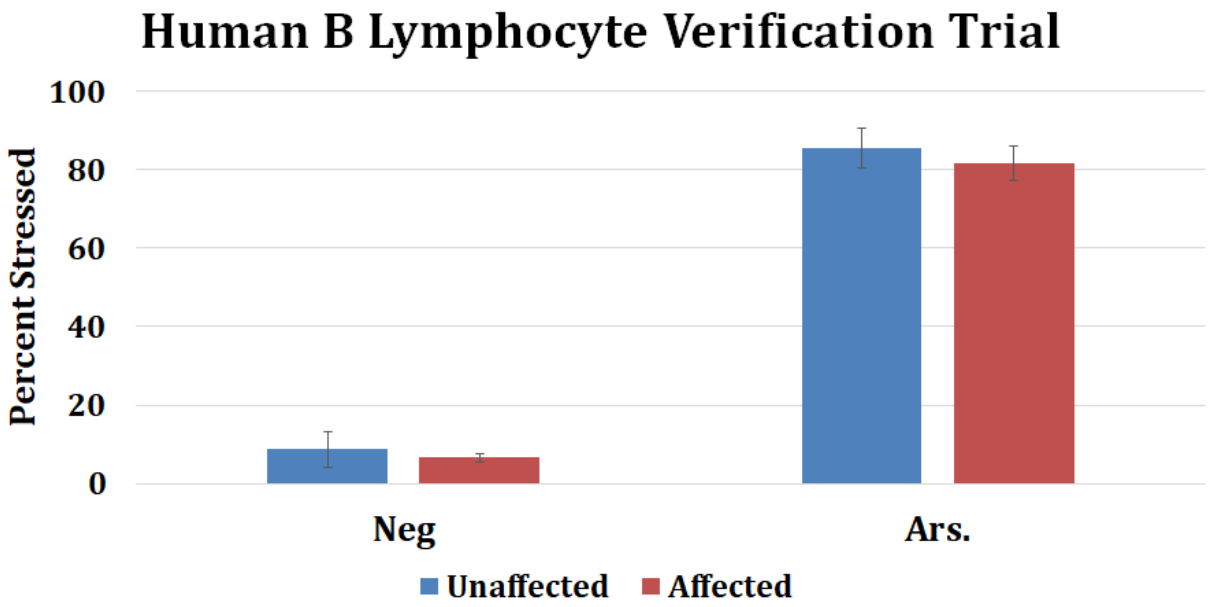

Figure 6. The average percentage of human B lymphocytes exhibiting stress. These cells were treated for one hour with 500 uM of arsenite or left untreated as indicated in the figure. One biological replicate was performed. Error bars within the figure represent standard error.

There is a clear difference between the untreated and arsenite treated B lymphocytes, which means these cells are a good candidate for the acute exposure assay. One biological replicate was performed but was counted by all four team members. The standard error shows the variance between the four counts.

Mouse Embryonic Fibroblasts

In order to determine if there was a difference in stress granule formation between the wild type (WT) and *FMR1* knockout (KO) mouse embryonic fibroblast (MEF) cell lines, an acute exposure assay to arsenite and BPA was performed. Identical to the verification trials, an untreated sample served as the negative control and an arsenite treated sample was the positive control. The fibroblasts were treated with varying concentrations of BPA and the treatment was left on the fibroblasts for one hour. The cells were then fixed, stained with anti-G3BP antibody, and scored for stress granules using fluorescence microscopy. Images taken using the fluorescent microscope can be seen in Figure 7. Panel A shows cells that are not considered to be stressed. Panel B shows cells that contain stress granules (the bright red dots).

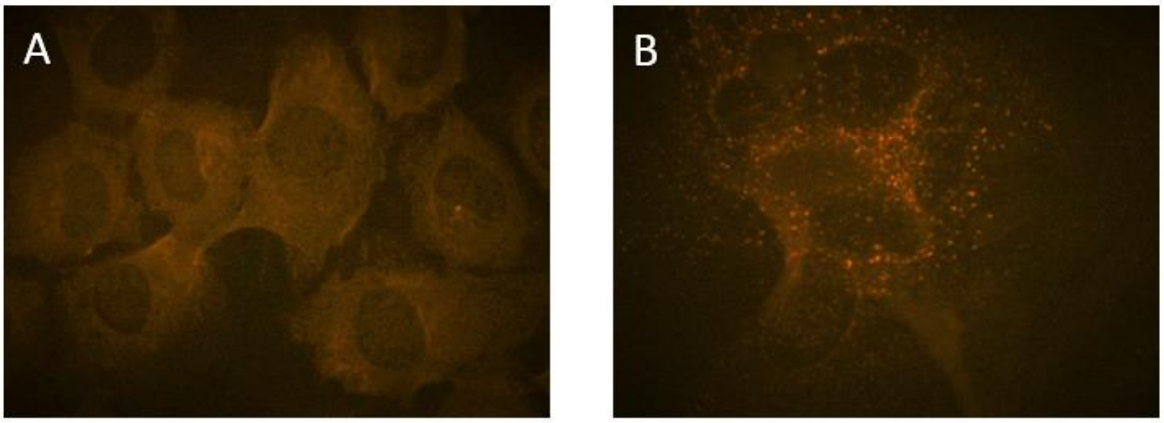

Figure 7. Images taken of mouse embryonic fibroblasts using fluorescence microscopy. (A) shows cells that do not have any stress granules and therefore are not considered to be stressed. (B) shows cells that have stress granules and there are considered to be stressed

The percentage of cells which had stress granules was calculated and depicted in Figure 8. Raw data for the experiment can be found in Appendix C.

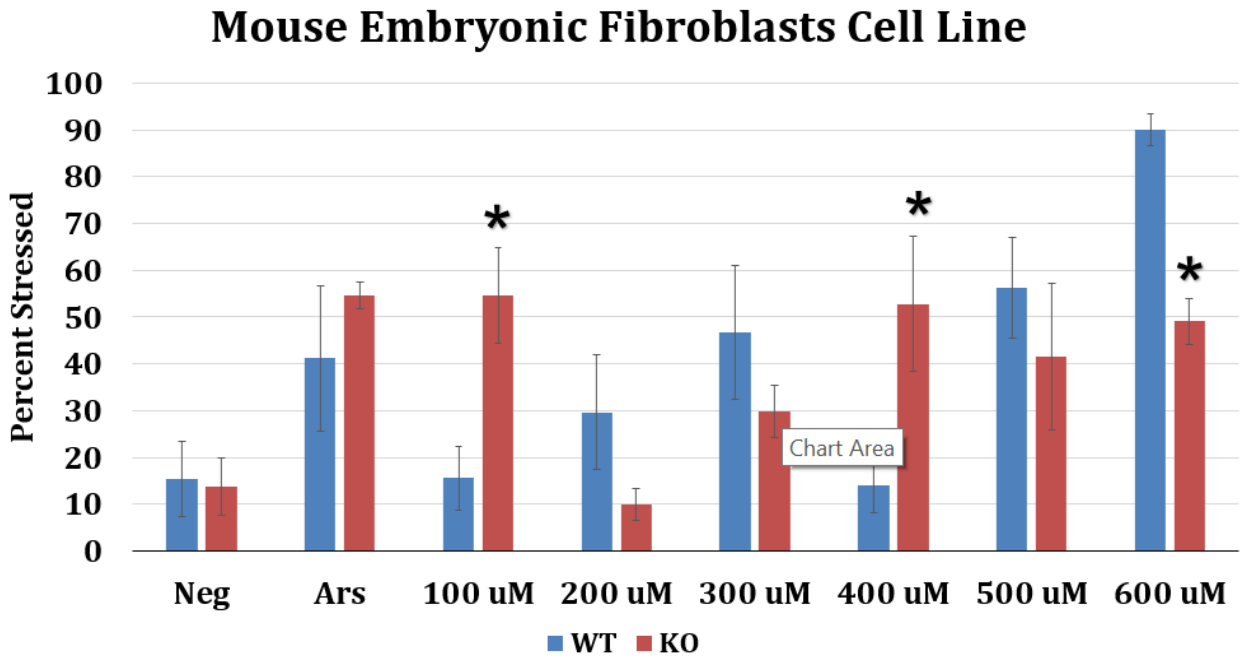

Figure 8. The average percentage of MEFs exhibiting stress. These cells were treated for one hour with varying concentrations of BPA, 500 uM of arsenite, or left untreated as indicated in the figure. Three biological replicate were performed. Error bars within the figure represent standard error. ANOVA analysis was completed to find statistical significance and is indicated by an asterisk ($p < 0.05$).

After graphing the average percent of stressed cells, there was no dose dependent response as previously predicted. For WT cells, the greatest percentage of stress was at the 600 uM concentration at 90%. The lowest percentage of stress was at the 400 uM concentration at 14% stress. For the KO cells, the highest percentage of stress occurred for the positive control and at 100 uM (54%) and the lowest percentage of stress occurred at 200 uM (10%). Three biological replicates were completed and each replicate was counted by two members for a grand total of six counts for each treatment. Standard error shows the variance in biological replicates from experiment to experiment. An ANOVA analysis was conducted to find if data from the WT and KO cell lines were statistically significant at each concentration. The data was found to be statistically significant at the 100 uM, 400 uM, and 600 uM concentration for a p value, $p < 0.05$.

Human B Lymphocytes

In order to determine if there was a difference in stress granule formation between the WT and FXS affected human B lymphocyte cell lines, an acute exposure assay to arsenite and BPA was performed. An untreated sample served as the negative control and an arsenite treated sample as the positive control. The lymphocytes were treated with varying concentrations of BPA and the treatment was left on the lymphocytes for one hour. The cells were then fixed, antibody stained, and scored for stress granules using a fluorescent microscope. Images of the cells taken by the fluorescent microscope can be seen in Figure 9.

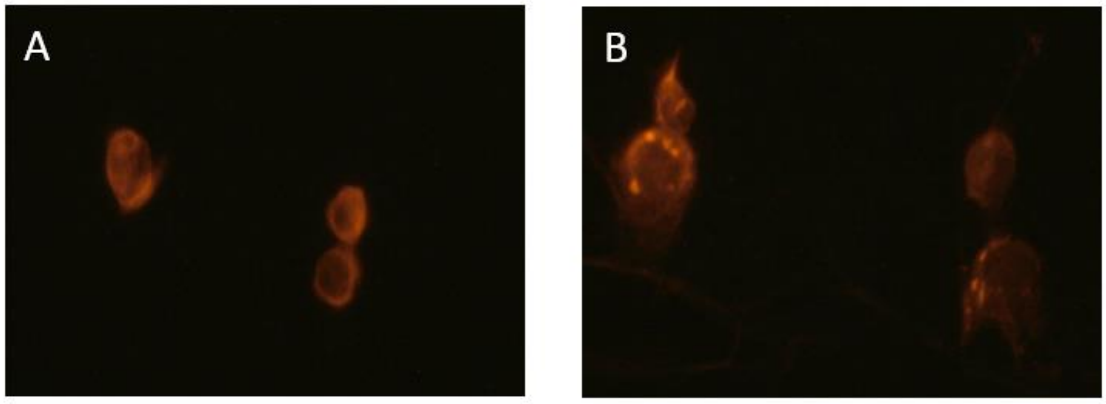

Figure 9. Images taken of human B lymphocytes using fluorescence microscopy. (A) shows cells that do not have any stress granules and therefore are not considered to be stressed. (B) shows cells that have stress granules and there are considered to be stressed

The data obtained from this experiment is shown in Figure 10. Raw data for the experiment can be found in Appendix D.

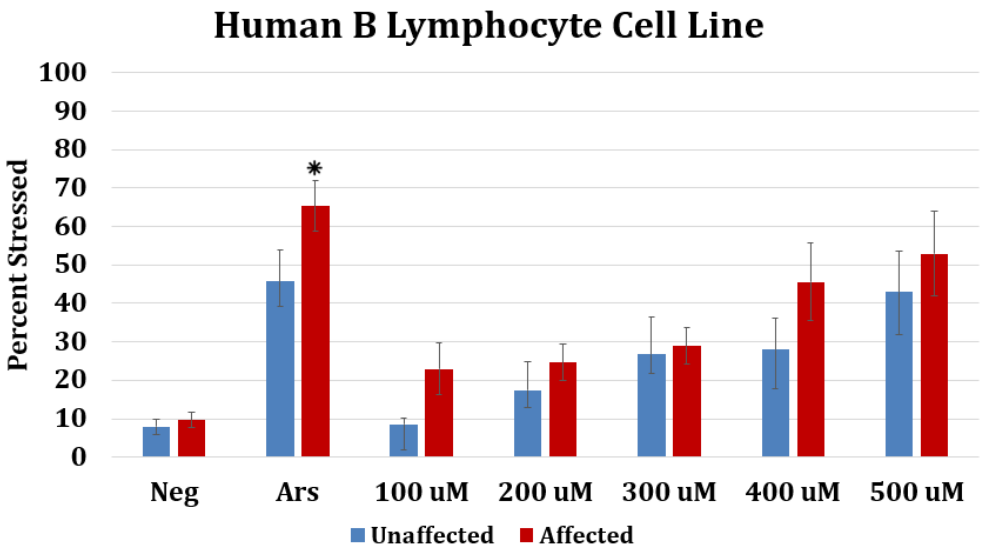

Figure 10. The average percentage of human B lymphocytes exhibiting stress. These cells were treated for one hour with varying concentrations of BPA, 500 uM of arsenite, or left untreated as indicated in the figure. Three biological replicate were performed. Error bars within the figure represent standard error. ANOVA analysis was completed to find statistical significance and is indicated by an asterisk ($p < 0.05$).

As seen in Figure 10, the data mimics the dose dependent response as predicted and seen in the U20S-DS cell line. There was an increase in percent of stressed cells as the concentration of BPA exposure increased. Three biological replicates were completed and each replicate was counted by two members for a grand total of six counts for each treatment. Standard error shows the variance in these counts. An ANOVA analysis was conducted to find if data from the unaffected and affected cell lines were statistically significant at each concentration. The data was found to be statistically significant for our positive control, Arsenite, only with a p value where $p < 0.05$.

Discussion

We noted a significant increase in stress granule formation in FXS affected B lymphocytes in response to arsenite. The FXS affected cells showed increased stress granule formation, however, this difference was not statistically significant. The results from the FXS affected mouse fibroblast cells had some statistical significance, but no conclusive findings. Further research is necessary to determine if there is a statistical significance in stress granule formation between FXS affected and unaffected cells.

Reasons for Discrepancies in Data

There were no trends seen in the mouse embryonic fibroblast data, which could be the result of many factors. These cells were the same type but they were not littermate and passage matched: they were not from mice from the same litter, nor were they at the same passage number and therefore they did not follow the same growth trends. These variable growth trends led to the unaffected cells undergoing more passages than the affected cells. This can affect their base level of stress, and therefore, alter the results after the treatment. If one group of cells is more stressed when plating one day over another, this would yield different results. Since we averaged all of the counts we did for each biological replicate, the day-to-day differences could have altered the overall result.

Additionally, some technical errors could have affected the results we obtained. While scoring the embryonic fibroblast cells, the microscope in Goddard Hall broke and required repair, so in order to keep the momentum of the project going, we used a microscope owned by the Biomedical Engineering Department in Salisbury Labs. This scope worked by taking photos of the cells and counting them on a computer screen, rather than looking through the eyepiece of the microscope to count. This change in methods may have altered our results.

Once the cells were mounted on the slides, the team took precautions so the slides would not be exposed to light for an extended amount of time, however, they still may have faded which would cause the fluorescently stained stress granules to appear dimmer, making the counter less likely to see them or count the cell as stressed.

Many of these problems were ameliorated when the team transitioned over to the B lymphocytes. The cells used for the B lymphocyte trials were from brothers: one has Fragile X Syndrome and one does not. This means that these cells were better matched, and they grew at about the same rate so they had about the same number of passages. However, discrepancies are seen between the verification trial and the results with these cells. The level of stress seen in the arsenite treated (positive control) is much higher, and not significantly different between cell types, for the verification trial. We predict that this is because the way this verification trial was set up led to subconscious bias to alter the results. The verification trial was not blinded. Additionally, we knew that the slides were either a positive or negative control, which could cause us to count cells as stressed when in a blinded count we would evaluate the cell more closely before counting it as stressed or unstressed.

Future Experimentation

This project grew out of previous experimentation evaluating cellular stress response to various concentrations of BPA. Therefore, we have some suggestions for future experiments with FXS affected cells and BPA. We recommend the following:

1. Redo the experiment with the mouse fibroblast cells, but remove some of the issues that we came across. Use matched cell lines if they are available, and try to be more consistent with passaging so the cells undergo the same number of passages. Use the same microscope throughout the experiment. Continue with performing biological replications in triplicate and perform at least two technical replicates to account for personal error.
2. Redo the experiment with B lymphocytes. These cells performed well for this team but assuring that the results are reproducible will add to the validity of the study. If this is the case, treat the cells with BPF or BPA and BPF to see if other bisphenols, or a combination of bisphenols, affects stress response.
3. Another worthwhile change would be to see how affected and wildtype cells react to incubation with arsenite or BPA at different incubation times. Stress granules are dynamic, and by incubating at different times there are more snapshots of the stress response available that would give information about reaction time to the compounds that could vary between affected and unaffected cells.

Impact on Patients with Fragile X Syndrome

If the results for these experiments are reproducible, they can be used to draw conclusions on how environmental contaminants may affect patients with FXS. For instance, if FXS patients are more sensitive to environmental contaminants, they may form stress granules at lower levels of exposure. If FXS patients' cells have an increased number of stress granules, the cells are not able to synthesize protein because translation is inhibited in stress granules. Therefore, the cell will eventually die, or work improperly, because it is not able to produce proteins. Chronic cell stress can cause ischemia, neurological diseases, and cancer (Reineke and Neilson, 2019).

Once information as to whether or not the differences in cellular stress response translate to differences at the patient level, further research can be done to see if the effects seen in patients with FXS are the same in patients with other ASD and intellectual and developmental disabilities.

Chapter Two: Disease and the Human Experience: ED Visits for Intellectually and Developmentally Disabled Patients Versus National Averages

Abstract

Research has found an altered stress response in cells with Fragile X Syndrome. Literature has shown that patients with intellectual and developmental disabilities (IDD) experience an increased rate of ED admissions. I hypothesized that IDD individuals would have different trends in their ED diagnoses due to the differing stress response. I conducted epidemiological research of a population of IDD individuals' ED visits compared to national averages and the infection and injury rates were found to be elevated.

Introduction

Although plastics and BPA have been shown to be present in different levels for those with certain intellectual and developmental disabilities (IDD), there is little verifiable research about how toxicity in general affects people with IDD. The biology portion of this MQP showed an increase in stress granule formation in human B lymphocytes affected with Fragile X Syndrome present with more stress granules when exposed to arsenite and BPA, two known toxins. Children with IDD have an increased risk of exposure to toxins like lead and mercury due to certain tendencies in behavior and communication. In addition, certain dietary restrictions like galactosemia may increase the effects of lead exposure for the child. If the child is exposed to toxins and shows symptoms they may present themselves similarly to the IDD and may be overlooked (Graft *et. al* 2006). However, there are many more problematic toxins than just mercury and lead.

Stress granules also form as a protective measure against stressors other than toxins, such as fever. People with IDD are more likely to have certain health problems with could lead cellular stress and to ED visits. The question persists regarding how this difference in stress granule formation may affect individuals at the patient level and contribute to differences in Emergency Department (ED) visits between people with IDD and the general public.

This study will analyze the ED visits of patients with IDD at Canton-Potsdam Hospital (CPH) and address possible reasons for the trends seen in this study population.

Epidemiology Studies

Epidemiology is defined by the Boston Medical Journal as "is the study of how often diseases occur in different groups of people and why". Studies like these give insight as to how certain groups are affected by diseases or conditions and the severity and frequency of their cases. In small scale studies where only one hospital is the source of patient data, the answer to why the differences (or lack thereof) are the way they are may not be evident or show a significant enough correlation to draw conclusions. Studies that show a group's health trends in different locations, under different conditions, and at different hospitals allow for conclusions to be drawn between the group and their health trends.

Health Among People with Intellectual and Developmental Disabilities

People with IDD have an increased risk of certain conditions, notably: "motor deficits, epilepsy, allergies, otitis media, gastroesophageal reflux disease (GERD), dysmenorrhea, sleep disturbances, seizure disorders, mental illness, vision and hearing impairments, oral health problems, and constipation" (May, *et. al*, 2010). Constipation affects 75% of people with IDD, GERD affects as many as 48%, 25% experience otitis media (middle ear infection), and 35-90% have sleep disturbances. These conditions can directly lead to visits to the ED, but also a person's reaction to the condition may also cause injury and send the person to the hospital. May *et. al* (2010) discussed that otitis media can lead to self-injury. This can escalate the problem to require an ED visit.

People with IDD use the ED 1.56-2.68x more than the general population according to reports from Canada and England (Hosking, *et. al*, 2017 & Lunsky *et. al*, 2012). These prevalence numbers were not found in published literature for the United States. One study conducted in the United States looked at the ED trends of patients with IDD and found that "intellectually

disabled adults were more likely ($P < .05$) than the convenience sample of the general adult ED population to have ICD-9 diagnoses among infectious/parasitic, nervous system/sense organs, and respiratory disorders, less likely among neoplastic, mental, circulatory, musculoskeletal, and injury/poisoning disorders" (Venkat *et. al*, 2011). The study done by Venkat *et. al* was conducted with patients from one residential care setting. For this MQP one hospital was used, and the living situation for the patients was not provided.

Objectives

This study will aim to evaluate the most common reasons people with IDD visit the Emergency Department by looking at a sample study population, their primary diagnoses in the Emergency Department, and the possible reasons this is the case. I predict that there will be differences in the diagnoses patients with IDD receive at CPH compared to national averages.

Materials and Methods

Obtaining Patient Data

Patient data was obtained after IRB approval from Worcester Polytechnic Institute (WPI) and Canton-Potsdam Hospital. It was pre-screened for relevance and all identifiable information was omitted prior to the release of it on the WPI password secure serve.

Included and Excluded Data

The patients that were included in the study data set were all diagnosed with intellectual and developmental disabilities, though the exact diagnosis was not provided, as it would cause the data to be more identifiable due to the small community this hospital serves.

Primary, secondary, and tertiary diagnoses for each emergency department visit were given in the data set; however, the primary diagnoses were the only one used, unless the primary diagnosis was a note and not a diagnosis, in which case the secondary diagnosis was used instead.

Based on these qualifications, a total of 82 patients and 400 individual visits to the Emergency Department of Canton-Potsdam Hospital (between October 2000 and January 2019).

National averages were obtained from National Hospital Ambulatory Medical Care Survey's Emergency Department Summary Tables. Years 2008-2015 were averaged in order to give a view of national averages over the eight-year period. These years were chosen because 2015 was the most recent data available from the CDC when this project was conducted and 2008 was the latest available that was provided in the same manner as previous years.

Data Mining

ICD-9 and ICD-10

The data set had diagnoses that were categorized by ICD-9 and ICD-10 coding. Since the national averages were done with ICD-9 information, all ICD-10 diagnoses in the study set were converted to ICD-9. This was done by converting groups (i.e. diseases of the respiratory system was a category in ICD-9 and ICD-10) but also individuals by searching the ICD-9 code on online databases when the categories were not as clearly transferable (i.e. ICD-9 paired "endocrine, nutritional and metabolic diseases, and immunity disorders" and "diseases of the blood and blood forming organs" where ICD-10 paired "diseases of the blood and blood forming organs and certain disorders involving the immune system" and "endocrine, nutritional, and metabolic diseases").

After this conversion was done, all data was used, evaluated, and displayed in ICD-9 coding.

Categorization

The categories used were predetermined ICD-9 codes. They include the following:

- Infectious and parasitic diseases
- Neoplasms
- Endocrine, nutritional and metabolic diseases, and immunity disorders
- Diseases of the blood and blood forming organs
- Mental disorders

- Diseases of the nervous system
- Diseases of the sense organs
- Diseases of the circulatory system
- Diseases of the respiratory system
- Diseases of the digestive system
- Diseases of the genitourinary system
- Diseases of the skin and subcutaneous tissue
- Diseases of the musculoskeletal system and connective tissue
- Symptoms, signs and ill-defined conditions
- Injury and poisoning*
- Supplementary classifications

*Further breakdown was available for injury and poisoning:

- Fractures
- Sprains and strains
- Intracranial injury
- Open wounds
- Superficial injury
- Contusion with intact skin surface
- Foreign bodies
- Burns
- Trauma complications and unspecified injuries
- Poisoning and toxic effects
- Surgical and medical complications
- Other

Data Analysis

Each encounter was used as an individual data point in the data analysis. If one patient had six visits on different days, each of those visits counted toward the associated diagnosis. However, if there were multiple entries in the system for one visit, they were only counted once.

Due to the nature of this experiment, statistical analysis could not be done. This is because the study data only came from one group, and this group consisted of 82 people, so a standard deviation and standard error could not be calculated.

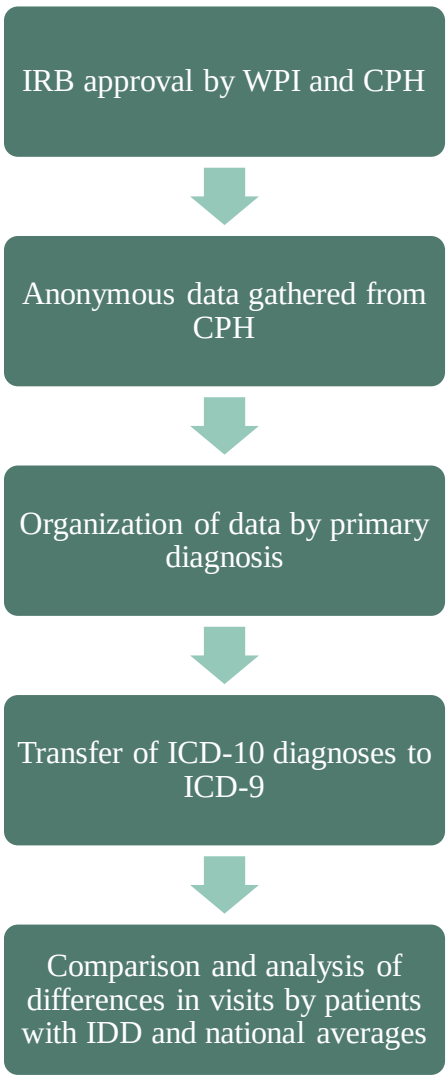

Figure 11. Visual Representation of the Methods of the Epidemiological Study

Results and Conclusions

Table 1. A comparison of the epidemiological findings and national averages provided by National Hospital Ambulatory Medical Care Survey (NHAMCS, 2008-2015)

Major Disease Category (ICD-9)	Epidemiological Findings	Average 2008-2015
Infectious and parasitic diseases	1.0%	3.0%
Neoplasms	0.0%	0.2%
Endocrine, nutritional, metabolic diseases, and immunity disorders	1.3%	1.6%
Mental disorders	0.8%	3.7%
Diseases of the nervous system and sense organs	5.0%	4.9%
Diseases of the circulatory system	1.5%	3.5%
Diseases of the respiratory system	13.3%	10.4%
Diseases of the digestive system	4.5%	6.0%
Diseases of the genitourinary system	5.8%	5.2%
Diseases of the skin and subcutaneous tissue	4.3%	3.9%
Diseases of the musculoskeletal system and connective tissue	3.0%	6.8%
Symptoms, signs and ill defined conditions	16.5%	22.3%
Injury and poisoning	39.0%	21.6%
Supplementary classifications	3.8%	2.6%

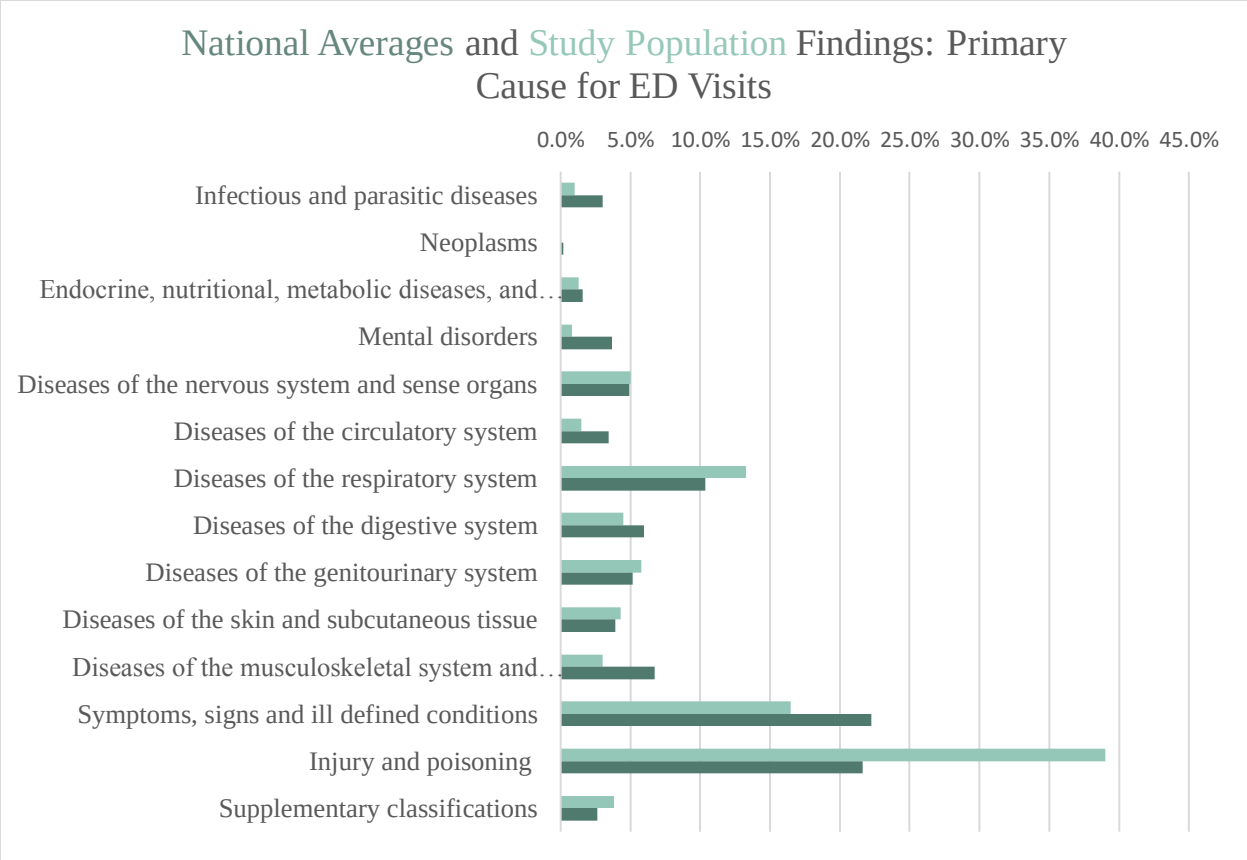

Figure 12. A comparison of the epidemiological findings and national averages provided by National Hospital Ambulatory Medical Care Survey (NHAMCS, 2008-2015)

This Figure shows the comparison between the study population and national averages for all main ICD-9 categories

The cumulative averages for five years of data (2008-2015) were compared to the epidemiological findings from the visits of patients with IDD at the ED.

Among the IDD population evaluated, injury and poisoning (39%) were the most common cause for the ED visits. In the national averages, “symptoms, signs, and ill-defined conditions” (22.3%) were most prevalent with injury and poisoning cases (21.6%) making up the second most diagnoses.

There was a further breakdown available in the national average tables for injury and poisoning visits. Breakdowns for other ICD-9 categories was not available from the national averages so they will not be discussed in this report.

Table 2. Breakdown of the injury and poisoning diagnoses in the study findings and National Hospital Ambulatory Medical Care Survey averages (NHAMCS, 2008-2015)

Injuries and Poisoning Subcategories	Study Population	Average 2008-2015
Fractures (800-829)	4.3%	2.8%
Sprains and Strains (840-848)	6.5%	4.4%
Intracrainal injury (850-854)	2.0%	0.4%
Open wounds (870-897)	8.5%	4.0%
Superficial injury (910-919)	2.5%	1.2%
Contusion with intact skin surface (920-924)	9.3%	3.3%
Foreign bodies (930-939)	1.0%	0.4%
Burns (940-949)	0.3%	0.4%
Trauma complications and unspecified injuries (958-959)	2.8%	2.0%
Poisioning and toxic effects (960-989)	0.3%	0.7%
Surgical and medical complications (996-999)	0.8%	0.4%
Other	1.0%	1.3%

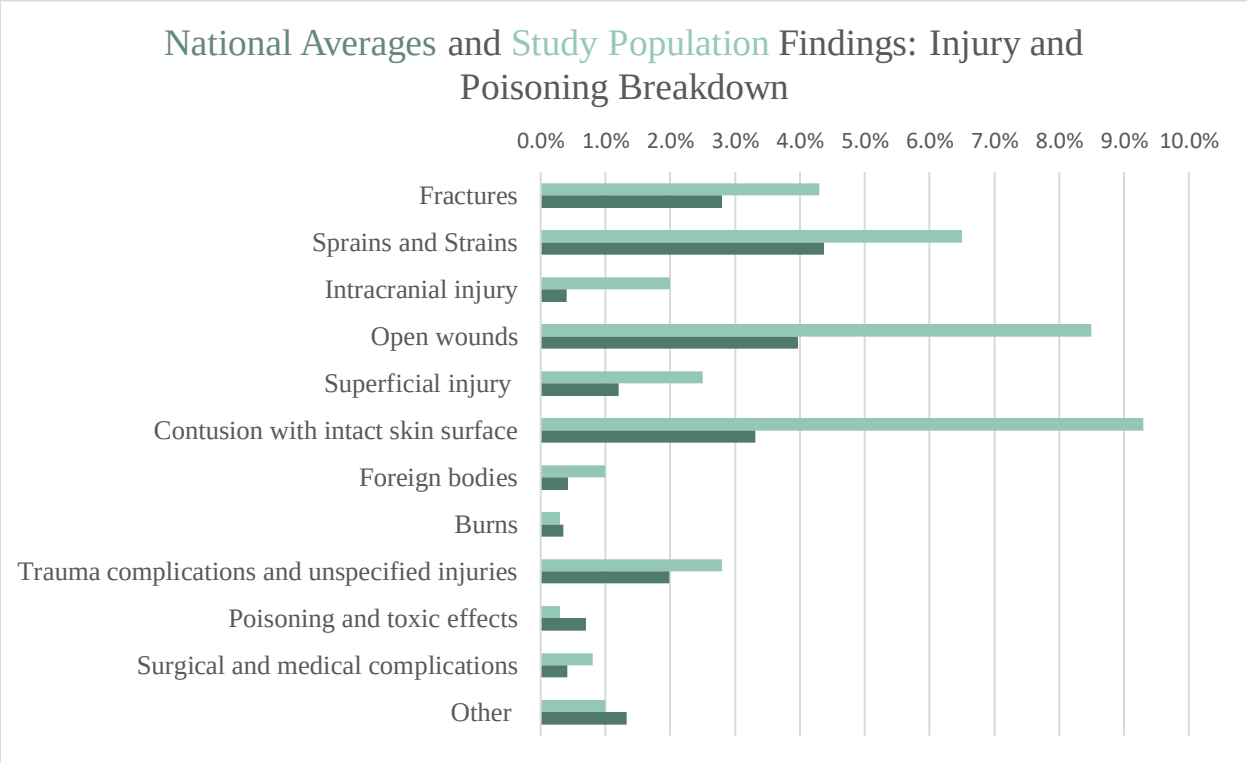

Figure 13. Breakdown of the injury and poisoning diagnoses in the study findings and National Hospital Ambulatory Medical Care Survey averages (NHAMCS, 2008-2015)

This Figure shows the comparison between the study population and national averages for the injury and poisoning category.

The breakdown of injury and poisoning diagnoses showed that contusions (9.3%) and open wounds (8.5%) were the most common injury and poisoning diagnoses for the IDD population. Strains and sprains (4.4%) were the most common nationally. It is important to note that these percentages are compared to all cases recorded (n=400 for the study population), not just in the injury and poisoning subcategory.

Poisoning and toxic effects (0.3%) caused the lowest number of cases in the IDD population, tied with burns. This is a little under half of the proportion of cases caused by poisoning and toxic effects in the general population (0.7%).

Overall, the prediction that IDD individuals may have significantly different diagnoses in the ED cases than the national averages was supported. However, due to the small sample size, more research needs to be done in order to draw conclusions for people with IDD as a whole.

Discussion

Although this study is small, there are some points that can be drawn from it especially for Canton-Potsdam Hospital. This study supports the general idea that people with IDD have different medical needs and use the ED for different things. These trends, if studied more, can be used to serve this population more efficiently.

Limitations

There are many limitations for a study this size. For one, the population this study worked off of was only 82 people with 400 total emergency department visits. This is not large enough to draw true conclusions, but it serves as a pilot study to look at trends and form goals for further research.

Additionally, there are factors that could contribute the chief complaint of the visit that is not necessarily tied to the patient's IDD but rather their living environment, elements of the climate, and the type of area (rural, suburban, urban) they live in. A larger population would allow for more conclusions to be drawn and for statistical analysis to be done to find out if the differences seen in ED visit trends are caused by IDD.

Causes for Differences in Trends

The trends seen in this study are in contrast to those seen in Venket *et. al*. The study done by Venket *et. al* found significantly less visits attributed to injury and poisonings. However, that study was done with people who were in residential care facilities, whereas this study was not limited to people from certain living situations and most likely was composed of individuals from residential care facilities and those who lived at home.

The variety in diagnoses for IDD individuals compared to the national averages could stem from a few things. Since the majority of injury and poisoning visits were caused by physical trauma related conditions (35%) these individuals may be more prone to these conditions based on their disability. May *et. al* (2010) notes that motor deficits, epilepsy, and seizure disorders are more prevalent in individuals with IDD. All of these conditions can lead to physical trauma. Additionally, if they live in a group home or under the supervision of a trained caretaker, they may be less likely to be exposed to things that may cause harm, like poisonous chemicals or alcohol which could lead to toxicity.

Further research

This study showed that there were differences in the reasons individuals with IDD came to the ED at CPH compared to national averages. Therefore, it is valuable to expand this study to see if these trends carry over with larger populations of people with IDD. Additionally, all of these visits occurred at Canton-Potsdam Hospital, a hospital in a St. Lawrence Country in Upstate New York. This is a rural location so this may influence the cases they see. Obtaining data from suburban, urban, and more rural hospitals from all states would add to the data pool and possibly allow for more insight into the trends of patients with IDD without the influence of location.

Additionally, if the population size allows for the breakdown of the patient's IDD diagnosis without causing issues with anonymity, research into individuals with specific diagnoses: Down Syndrome, Fragile X Syndrome, etc. would add to the specificity of the research and provide additional insights.

The research done here can help Canton-Potsdam Hospital improve the care of their patients with IDD; further research could guide healthcare providers in allocating resources and improving care in the ED for patients with IDD in all hospitals.

Connection to Science Writing

This project was an epidemiological study connecting disease and the human experience. Understanding the multi-faceted nature of health is essential for medical and scientific communication that benefits patients and the healthcare system. Additionally, science writing is fact-based, so science writers need to understand the difference between correlation and causation. This study only showed correlations because of the small sample of patients, so many different reasons for this were discussed, and causation was not drawn from the study.

References

Anderson P, Kedersha N. 2008. Stress granules: the Tao of RNA triage. Trends in Biochemical Sciences. 33(3):141-150.

Anderson P, Kedersha N. 2009. Stress granules. Cell Press. 19(10).

Antonia M. Calafat, Xiaoyun Ye, Lee-Yang Wong, John A. Reidy, Larry L. Needham. 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environmental Health Perspectives. 116(1):39-44.

Chen M, Ike M, Fujita M. 2002. Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. Environmental Toxicology. 17(1):80-6.

Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraiser CE, Stone EF, Chen C, Fak JJ, Licatalosi DD, et al. 2011. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146:247–261.

Davidovic L, Navratil V, Bonaccorso CM, Catania MV, Bardoni B, Dumas M. 2011. A metabolomic and systems biology perspective on the brain of the fragile X syndrome mouse model. Genome Research. 21:2190-202.

Dobra I, Pankivskyi S, Samsonova A, Pastre D, Hamon L. 2018. Relation Between Stress Granules and Cytoplasmic Protein Aggregates Linked to Neurodegenerative Diseases. Current Neurology and Neuroscience Reports. 18(12):107

Epidemiology for the uninitiated. The BMJ. https://www.bmj.com/about-bmj/resources-readers/publications/epidemiology-uninitiated

Friend C, Hoppe M, Wu J, Farny N. 2018. Stressed Out! Effects of Bisphenols on the Cellular Stress Response. Worcester, MA. Worcester Polytechnic Institute.

Fukui, Y. S., Yumura, S., Yumur, T. K. 1987. Agar-overlay immunofluorescence: high resolution of cytoskeletal components and their changes during chemotaxis. Methods Cell Biol. 28:347–356.

Gareau C, Houssin E, Martel D, Coudert L, Mellaoui L, Huot M-E, Laprise P, Mazroui R. 2013. Characterization of fragile X mental retardation protein recruitment and dynamics in drosophila stress granules. PLoS One. 8(2):e55342.

Hall DA, Berry-Kravis E. 2018. Fragile X syndrome and fragile X-associated tremor ataxia syndrome. Handbook of Clinical Neurobiology. 147:377-91.

Hosking FJ, Carey IM, Dewilde S, Harris T, Beighton C, Cook DG. 2017. Preventable Emergency Hospital Admissions Among Adults With Intellectual Disability in England. The Annals of Family Medicine 15:462–470.

Kedersha N, Stoecklin G, Ayodele M, Yacono P, Lykke-Andersen J, Fritzler MJ, Scheuner D, Kaufman RJ, Golan DE, Anderson P. 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J Cell Biol. 169(6):871.

Kedersha N, & Anderson P. 2008. Stress granules: the Tao of RNA triage. Trends Biochem Sci. 33(3): 141-150

Konieczna A, Rutkowska A, Rachoń D. 2015. Health risk of exposure to bisphenol A (BPA). Roczniki Panstwowego Zakladu Higieny. 66(1):5.

Krishnan AV, Stathis P, Permuth SF, Tikes L, Feldman D. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. 1993;132:2279–2286

Kültz D. 2005. Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol. 67(1):225-57.

Lunsky Y, Balogh R, Khodaverdian A, Elliott D, Jaskulski C, Morris S. 2012. A Comparison of Medical and Psychobehavioral Emergency Department Visits Made by Adults with Intellectual Disabilities. Emergency Medicine International 2012:1–6.

Lunsky Y, Lin E, Balogh R, Klein-Geltink J, Wilton AS, Kurdyak P. 2012. Emergency Department Visits and Use of Outpatient Physician Services by Adults with Developmental Disability and Psychiatric Disorder. The Canadian Journal of Psychiatry 57:601–607.
May ME, Kennedy CH. 2010. Health and Problem Behavior Among People With Intellectual Disabilities. Behavior Analysis in Practice 3:4–12.
Mazroui R, Huot M, Tremblay S, Filion C, Labelle Y, Khandjian EW. 2002. Trapping of messenger RNA by fragile X mental retardation protein into cytoplasmic granules induces translation repression. Human Molecular Genetics. 11(24):3007-17.
National Hospital Ambulatory Medical Care Survey, 2008-2015. NHAMCS.
Panas M, Kedersha N, & Mcinerney G. 2015. Methods for the characterization of stress granules in virus infected cells. Methods, *90*, 57–64.
Poljšak B, Milisav I. 2012. Clinical implications of cellular stress responses. Bosnian Journal of Basic Medical Sciences. 12(2):122-6.
Protter D, Parker R. 2016. Principles and properties of stress granules. Cell Press. 26(9).
Reineke L, and Neilson, J. 2019. Differences between acute and chronic stress granules, and how these differences may impact function in human disease. Biochemical Pharmacology. 162:123-131
Rezg R, El-Fazaa S, Gharbi N, Mornagui B. 2014. Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. Environment International. 64:83-90.
Ritter SK. 2011. Bisphenol a. Chemical & Engineering News Archive. 89(23):13-22.
Rochester JR. 2013. Bisphenol A and human health: A review of the literature. Reprod Toxicol. 42:132-55.
Venkat A, Pastin RB, Hegde GG, Shea JM, Cook JT, Culig C. 2011. An analysis of ED utilization by adults with intellectual disability. The American Journal of Emergency Medicine 29:401–411.
Vogel SA. 2009. The politics of plastics: The making and unmaking of bisphenol A "safety". American Journal of Public Health. 99(S3):S566.

Appendices

Appendix A - Raw Data for U20S-DS cell line verification trial

Treatment	Stressed	Not Stressed	Total	% Stressed
Neg	1	258	259	0.39
	22	252	274	8.03
	7	258	265	2.64
	14	255	269	5.20
Ars	254	1	255	99.61
	303	0	303	100.00
	420	44	464	90.52
	268	1	269	99.63
100 uM	7	262	269	2.60
	18	257	275	6.55
	1	271	272	0.37
	18	274	292	6.16
200 uM	7	246	253	2.77
	16	302	318	5.03
	3	251	254	1.18
	16	248	264	6.06
300 uM	143	133	276	51.81
	140	137	277	50.54
	142	144	286	49.65
	112	146	258	43.41
400 uM	263	5	268	98.13

	58	**329**	**387**	**14.99**
	249	**10**	**259**	**96.14**
	255	**89**	**344**	**74.13**
500 uM	**249**	**4**	**253**	**98.42**
	268	**5**	**273**	**98.17**
	255	**14**	**269**	**94.80**
	291	**113**	**404**	**72.03**

Appendix B - Raw Data for Human B Lymphocyte verification trial

Unaffected						Affected				
Treatment	Stressed	Not Stressed	Total	Percent Stressed		Treatment	Stressed	Not Stressed	Total	Percent Stressed
Neg	4	177	181	2.21		Neg	14	191	205	6.83
	3	104	107	2.80			8	139	147	5.44
	3	119	122	2.46			11	127	138	7.97
	44	169	213	20.66			5	168	173	2.89
Ars	150	13	163	92.02		Ars	189	24	213	88.73
	104	12	116	89.66			78	35	113	69.03
	113	9	122	92.62			123	22	145	84.83
	116	47	163	71.17			164	44	208	78.85

Appendix C - Raw Data for MEF cell line acute exposure assay

Wildtype					Knockout				
Treatment	Stressed	Not Stressed	Total	Percent Stressed	Treatment	Stressed	Not Stressed	Total	Percent Stressed
Neg	1	255	256	0.39	Neg	38	96	134	28.36
	6	232	238	2.52		41	69	110	37.27
	71	116	187	37.97		7	243	250	2.80
	89	116	205	43.41		11	247	258	4.26
	9	250	259	3.47		6	216	222	2.70
	11	260	271	4.06		16	198	214	7.48
Ars	20	241	261	7.66	Ars	138	80	218	63.30
	24	235	259	9.27		117	116	233	50.21
	248	18	266	93.23		89	108	197	45.18
	17	215	232	7.33		118	77	195	60.51
	165	126	291	56.70		145	108	253	57.31
	218	81	299	72.91		130	124	254	51.18
100 uM	61	155	216	28.24	100 uM	60	153	213	28.17
	62	152	214	28.97		64	131	195	32.82
	17	245	262	6.49		159	76	235	67.66
	9	256	265	3.40		140	79	219	63.93
200 uM	148	133	281	52.67	200 uM	20	221	241	8.30
	163	173	336	48.51		43	170	213	20.19
	14	245	259	5.41		16	248	264	6.06
	27	197	224	12.05		11	205	216	5.09
300 uM	225	29	254	88.58	300 uM	30	226	256	11.72
	221	30	251	88.05		40	223	263	15.21
	46	204	250	18.40		71	159	230	30.87

	15	253	268	5.60		112	135	247	45.34
	109	139	248	43.95		95	137	232	40.95
	97	177	274	35.40		70	129	199	35.18
400 uM	105	172	277	37.91	400 uM	81	89	170	47.65
	9	243	252	3.57		114	66	180	63.33
	40	253	293	13.65		47	195	242	19.42
	72	218	290	24.83		14	215	229	6.11
	4	276	280	1.43		199	37	236	84.32
	7	209	216	3.24		242	10	252	96.03
500 uM	237	48	285	83.16	500 uM	227	39	266	85.34
	213	47	260	81.92		233	24	257	90.66
	48	147	195	24.62		51	207	258	19.77
	47	147	194	24.23		27	257	284	9.51
	157	102	259	60.62		95	137	232	40.95
	149	87	236	63.14		6	262	268	2.24
600 uM	242	37	279	86.74	600 uM	144	123	267	53.93
	236	17	253	93.28		115	145	260	44.23

Appendix D - Raw Data for Human B Lymphocyte cell line acute exposure assay

Unaffected					Affected				
Treatment	Stressed	Not Stressed	Total	Percent Stressed	Treatment	Stressed	Not Stressed	Total	Percent Stressed
Neg	7	111	118	5.93	Neg	7	126	133	5.26
	24	115	139	17.27		19	139	158	12.03
	12	166	178	6.74		30	183	213	14.08
	6	145	151	3.97		3	117	120	2.50
	12	186	198	6.06		15	198	213	7.04
	9	88	97	9.28		22	133	155	14.19
Ars	127	126	253	50.20	Ars	165	52	217	76.04
	81	62	143	56.64		100	78	178	56.18
	102	156	258	39.53		201	78	279	72.04
	93	124	217	42.86		142	135	277	51.26
	108	57	165	65.45		114	87	201	56.72
	9	91	100	9.00		102	7	109	93.58
100	26	232	258	10.08	100	97	115	212	45.75
	14	140	154	9.09		90	158	248	36.29
	11	116	127	8.66		13	109	122	10.66
	13	97	110	11.82		7	102	109	6.42
	21	171	192	10.94		27	208	235	11.49
	1	154	155	0.65		14	139	153	9.15
200	23	118	141	16.31	200	31	122	153	20.26
	26	148	174	14.94		35	112	147	23.81
	2	258	260	0.77		16	171	187	8.56
	35	201	236	14.83		34	129	163	20.86
	63	59	122	51.64		77	121	198	38.89

	32	69	101	31.68			44	72	116	37.93
300	8	137	145	5.52		300	37	109	146	25.34
	0	140	140	0.00			25	165	190	13.16
	12	204	216	5.56			41	114	155	26.45
	97	125	222	43.69			39	98	137	28.47
	129	99	228	56.58			107	109	216	49.54
	51	109	160	31.88			48	135	183	26.23
400	24	176	200	12.00		400	149	64	213	69.95
	11	154	165	6.67			105	75	180	58.33
	78	196	274	28.47			43	219	262	16.41
	95	171	266	35.71			20	95	115	17.39
	41	79	120	34.17			50	63	113	44.25
	69	40	109	63.30			96	36	132	72.73
500	103	49	152	67.76		500	98	22	120	81.67
	72	71	143	50.35			148	46	194	76.29
	24	67	91	26.37			22	109	131	16.79
	74	13	87	85.06			31	80	111	27.93
	47	154	201	23.38			40	74	114	35.09
	37	119	156	23.72			105	64	169	62.13