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A Survey Investigation of Hurricane Preparedness in New Orleans Post Hurricane Katrina

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Patrick F. Taylor Foundation

Introduction

In the summer of 2005, Hurricane Katrina made landfall in the Gulf Coast of the United States. The hurricane hit New Orleans on August 29. New Orleans, with the Mississippi River on one side and Lake Pontchartrain on the other, and with parts of the city below sea level ,is a place of risk. When the hurricane hit, some of the levees that protected the city breached, which led to flooding that left an estimated 80% of the city underwater. The catastrophic aftermath of Hurricane Katrina included property damage of almost $100 billion and loss of life for nearly 2000 people. A significant portion of people living in the City did not evacuate despite warnings. Many failed to do so because of lack of information or proper planning.

Almost 10 years have passed since Hurricane Katrina, but its memory is still very much alive. Many considerate that as long as the memory remains in the minds of the residents of New Orleans, the aftermath and catastrophic consequences cannot be repeated; however, many others do believe the city is not yet completely prepared.

We hypothesized that those who survived Katrina would be more likely to be prepared in subsequent hurricane seasons; that those with higher health literacy are more likely to be prepared; and that prior evacuation status would have an impact on current preparedness.

Objectives

- To assess the level of hurricane preparedness post hurricane Katrina among various levels of emergency department health care workers and their patients currently living in New Orleans.
- To assess what factors if any affect people's level of preparation for a hurricane and mandatory evacuation.
- To assess awareness of the plans put in place by the city for the contingency of a mandatory evacuation.

Methods

125 surveys were administered in the Emergency Department (ED) of the Interim LSU Hospital to a convenience sample of five populations of interest: staff physicians, residents, nurses, ancillary staff, and patients (25 surveys to each population) on weekday, night and weekend shifts to assess their awareness of city preparedness planning, their own level of preparation and evacuation plans, and their evacuation status during Katrina.

Multivariate analysis was conducted by survey item, using chi square test to correlate responses to population of interest as a surrogate for health literacy and to evacuation status during Katrina.

LSU HSC staff helps patients to evacuate

Survey Results

1	Resident during Katrina
2	Lost any family or friends to Katrina
3	Was prepared for the hurricane
4	Evacuated before mandatory evacuation
5	Evacuated after mandatory evacuation
6	Had transportation to evacuate the city
7	Had an evacuation location
8	Would evacuate with a recommended evacuation warning
9	Would evacuate with a mandatory evacuation warning
10	Has a hurricane preparedness kit
11	Has an evacuation plan
12	Has transportation to evacuate
13	Is registered for city-assisted evacuation
14	Is familiar with the city's 17 pick-up locations
15	Would be willing to evacuate using city assisted evacuation
16	Is aware of the city's mandatory evacuation plan
17	Is familiar with the website http://nola.gov/ready
18	Is signed up for the city's emergency notifications
19	Is aware of the city's hurricane ready website

Table 1. Questions asked during the survey

Figure 1. Attending Physician's Answers to the Survey

Figure 2. Residents' Answers to the Survey

Figure 3. Nurses' Answers to the Survey

Figure 4. Ancilary Staff's Answers to the Survey

Figure 5. Medical Students' Answers to the Survey

Figure 6. Patient's or Visitor's Answers to the Survey

Special thanks to Victoria Serven, Ferralita Madere, Preston Edwards, Deidra Jessie and Andrew Delrio for their assistance with this project.

Correlation Between Health Literacy and Survey Outcome

Figure 7. Association Between Health Literacy Level and Katrina Evacuation Status and Awareness of City Preparedness Planning

Figure 8. Association Between Health Literacy Level and Katrina Evacuation Status and Evacuation Planning

Figure 9. Association Between Health Literacy Level and Katrina Evacuation Status and Willingness to Evacuate with Recommended warning

Figure 10. Association Between Health Literacy Level and Katrina Evacuation Status and Willingness to Evacuate with Mandatory Warning

Conclusions

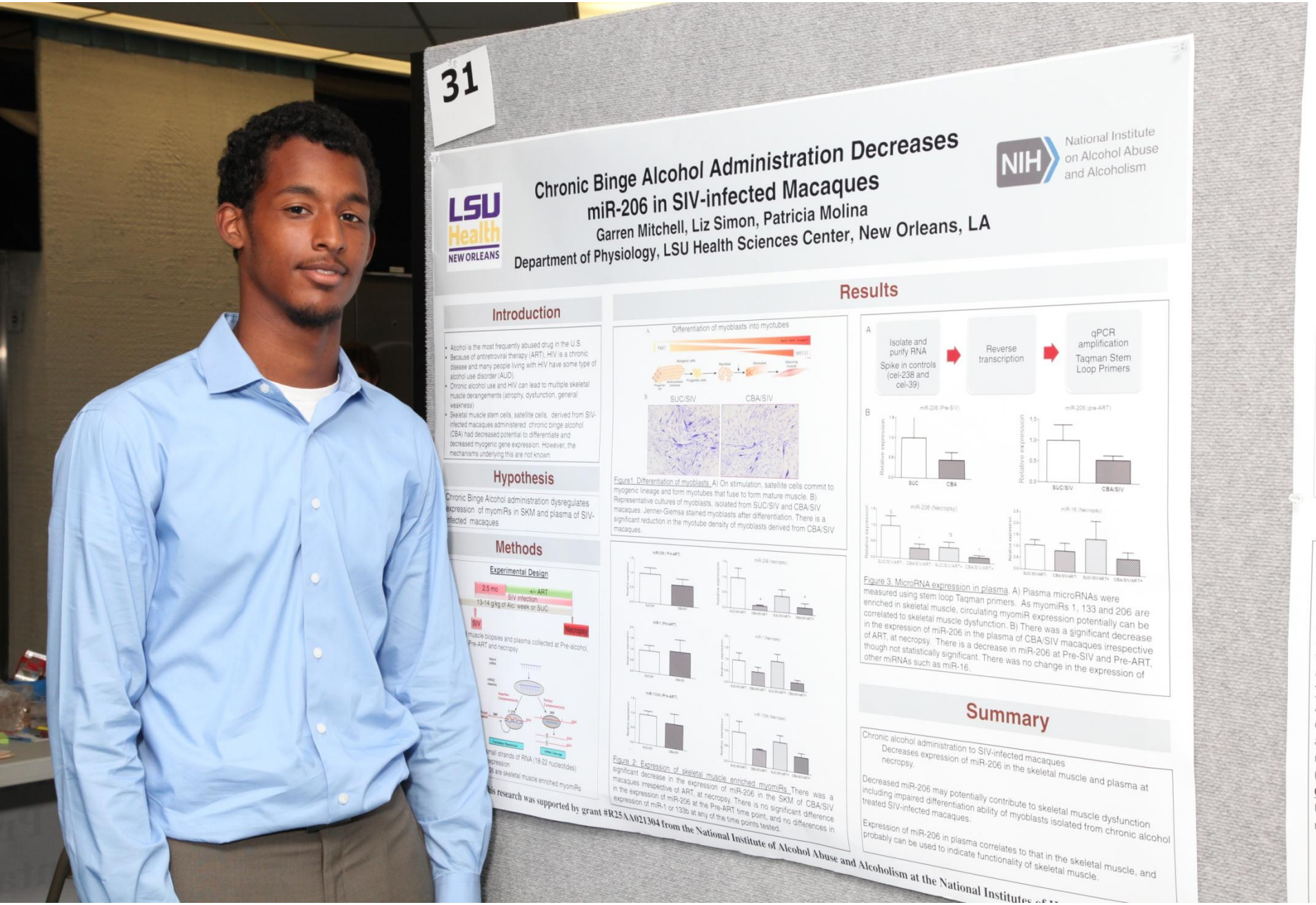
Health literacy level does not correlate to preparation for storms prr hurricane Katrina, but it does have an impact on the level of awareness for future preparedness and future evacuation. Katrina evacuation status also has a significant impact on future preparedness and future evacuation. Most of the people surveyed were not aware of the city's planning in case of emergency, which could imply it is not being well advertised.

Discussion

Level of training as a surrogate for health literacy did not correlate with preparation for previous storms. ED nurses indicated better preparedness in most categories, followed by ancillary staff. However, further analysis shows that health literacy is related to preparation for future storms, with attending physicians being the group best prepared. Having been through Katrina has an impact on the likelihood of better preparation for future storms.

Although attending physicians and residents would be willing to evacuate the city in case of recommended or mandatory evacuation, many stated they may be forced to stay in the city due to their responsibilities in the ED. Future studies should incorporate a question on whether professional duties impact a respondent's evacuation plans.

The City of New Orleans bears a special risk during tropical storms, making hurricane preparedness a very important consideration for citizens and for government. The correlations found in this study can be helpful in determining what is needed to better prepare the citizens of New Orleans for such an emergency.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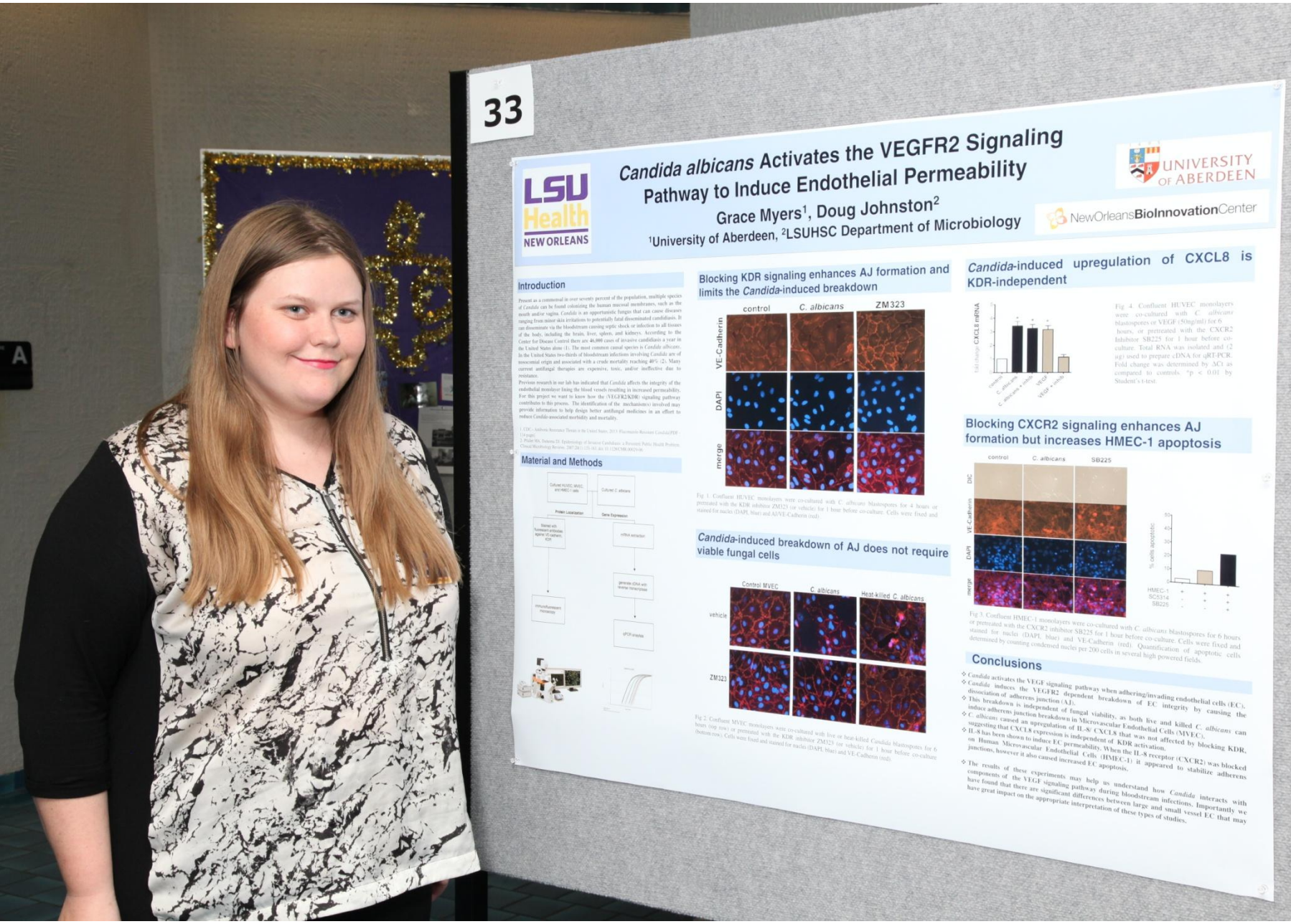
Chronic Binge Alcohol Administration Decreases miR-206 in SIV-infected Macaques
Garren Mitchell, Liz Simon, Patricia Molina
Department of Physiology, LSU Health Sciences Center, New Orleans, LA
NIH National Institute on Alcohol Abuse and Alcoholism
Introduction
Alcohol is the most frequently abused drug in the U.S.
Because of antiretroviral therapy (ART), HIV is a chronic disease and many people living with HIV have some type of alcohol use disorder (AUD).
Chronic alcohol use and HIV can lead to multiple skeletal muscle derangements (atrophy, dysfunction, general weakness)
Skeletal muscle stem cells, satellite cells, derived from SIV-infected macaques administered chronic binge alcohol (CBA) had decreased potential to differentiate and decreased myogenic gene expression. However, the mechanisms underlying this are not known
Hypothesis
Chronic Binge Alcohol administration dysregulates expression of myomiRs in SKM and plasma of SIV-infected macaques
Methods
Experimental Design
2.5 mo
+/- ART
SIV infection
13-14 g/kg of EtOH/week or SUC
SIV
Necropsy
muscle biopsies and plasma collected at Pre-alcohol, Pre-ART and necropsy
mall strands of RNA (18-22 nucleotides)
expression
are skeletal muscle enriched myomiRs
Results
Differentiation of myoblasts into myotubes
SUC/SIV
CBA/SIV
Figure1. Differentiation of myoblasts. A) On stimulation, satellite cells commit to myogenic lineage and form myotubes that fuse to form mature muscle. B) Representative cultures of myoblasts, isolated from SUC/SIV and CBA/SIV macaques. Jenner-Giemsa stained myoblasts after differentiation. There is a significant reduction in the myotube density of myoblasts derived from CBA/SIV macaques.
Figure 2. Expression of skeletal muscle enriched myomiRs. There was a significant decrease in the expression of miR-206 in the SKM of CBA/SIV macaques irrespective of ART, at necropsy. There is no significant difference in the expression of miR-206 at the Pre-ART time point, and no differences in expression of miR-1 or 133b at any of the time points tested.
Isolate and purify RNA
Spike in controls (cel-238 and cel-39)
Reverse transcription
qPCR amplification
Taqman Stem Loop Primers
miR-206 (Pre-SIV)
miR-206 (pre-ART)
miR-206 (Necropsy)
miR-16 (Necropsy)
Relative expression
SUC
CBA
SUC/SIV
CBA/SIV
Figure 3. MicroRNA expression in plasma. A) Plasma microRNAs were measured using stem loop Taqman primers. As myomiRs 1, 133 and 206 are enriched in skeletal muscle, circulating myomiR expression potentially can be correlated to skeletal muscle dysfunction. B) There was a significant decrease in the expression of miR-206 in the plasma of CBA/SIV macaques irrespective of ART, at necropsy. There is a decrease in miR-206 at Pre-SIV and Pre-ART, though not statistically significant. There was no change in the expression of other miRNAs such as miR-16.
Summary
Chronic alcohol administration to SIV-infected macaques
Decreases expression of miR-206 in the skeletal muscle and plasma at necropsy.
Decreased miR-206 may potentially contribute to skeletal muscle dysfunction including impaired differentiation ability of myoblasts isolated from chronic alcohol treated SIV-infected macaques.
Expression of miR-206 in plasma correlates to that in the skeletal muscle, and probably can be used to indicate functionality of skeletal muscle.
is research was supported by grant #R25AA021304 from the National Institute of Alcohol Abuse and Alcoholism at the National Institutes of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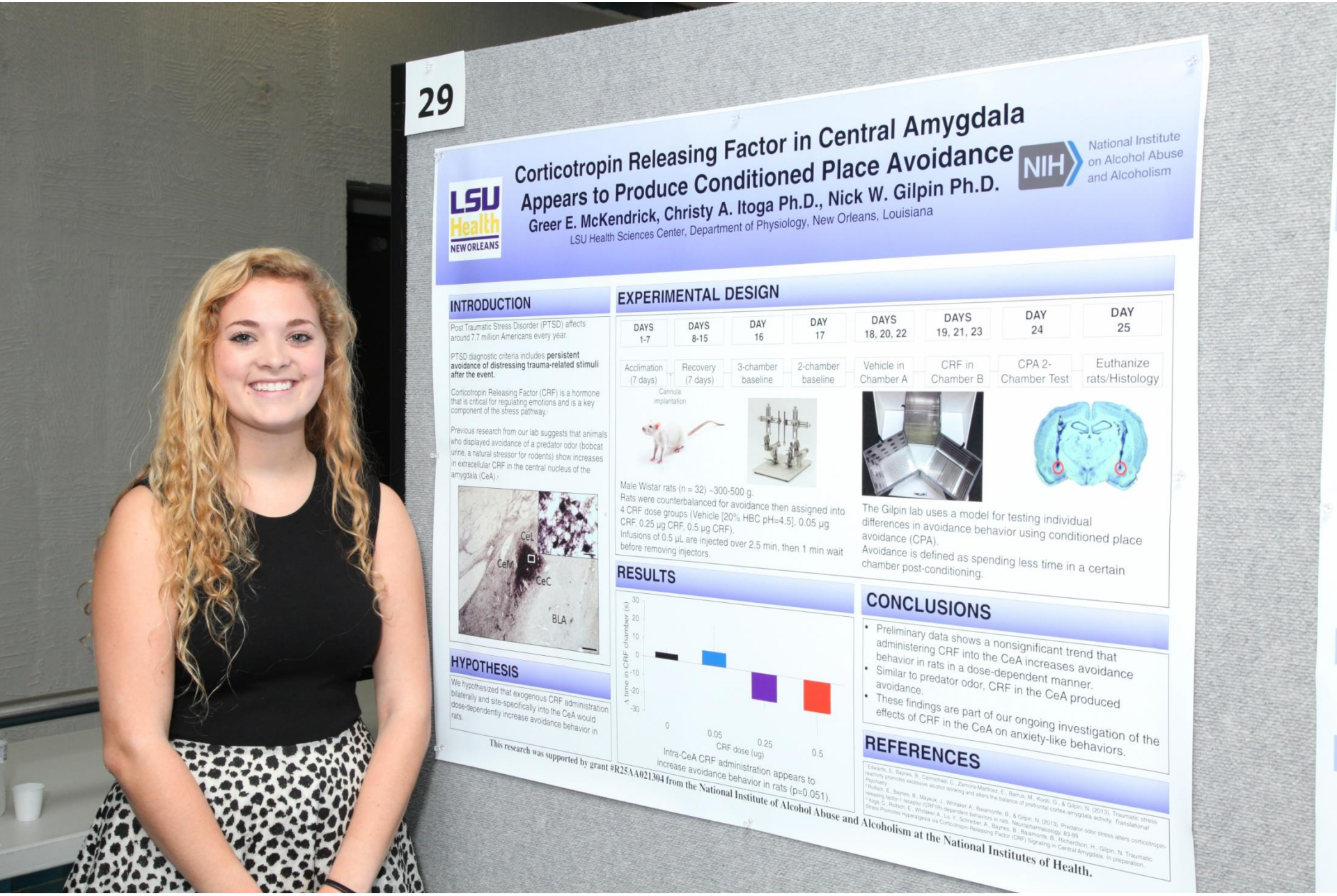
UNIVERSITY OF ABERDEEN
NewOrleansBioInnovationCenter
Candida albicans Activates the VEGFR2 Signaling Pathway to Induce Endothelial Permeability
Grace Myers[1], Doug Johnston[2]
[1]University of Aberdeen, [2]LSUHSC Department of Microbiology
Introduction
Material and Methods
Blocking KDR signaling enhances AJ formation and limits the Candida-induced breakdown
control
C. albicans
ZM323
VE-Cadherin
DAPI
merge
Candida-induced breakdown of AJ does not require viable fungal cells
Control MVEC
C. albicans
Heat-killed C. albicans
vehicle
ZM323
Candida-induced upregulation of CXCL8 is KDR-independent
Blocking CXCR2 signaling enhances AJ formation but increases HMEC-1 apoptosis
control
C. albicans
SB225
DIC
VE-Cadherin
DAPI
merge
% cells apoptotic
HMEC-1
SC3314
SB225
Conclusions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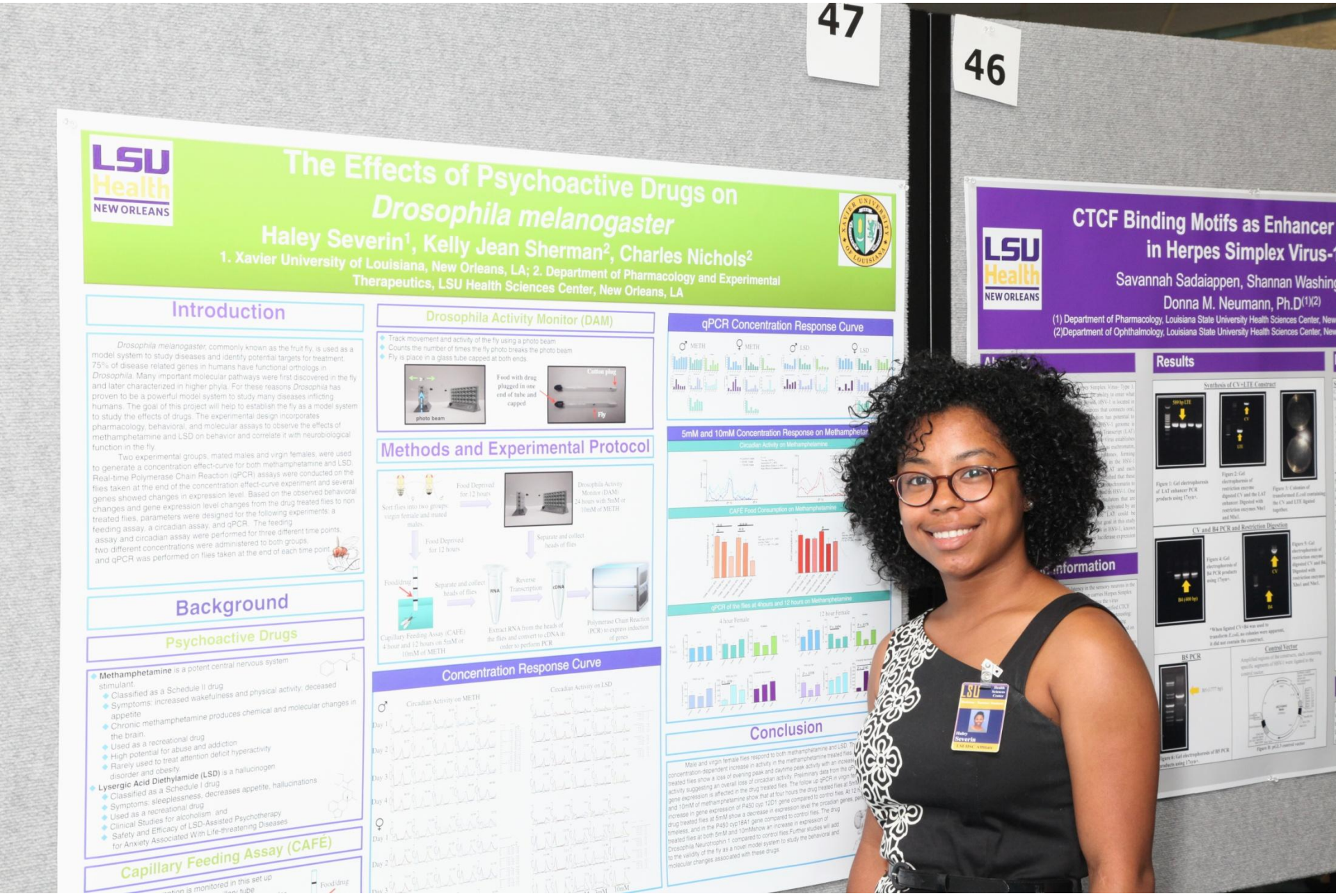
Corticotropin Releasing Factor in Central Amygdala Appears to Produce Conditioned Place Avoidance
Greer E. McKendrick, Christy A. Itoga Ph.D., Nick W. Gilpin Ph.D.
LSU Health Sciences Center, Department of Physiology, New Orleans, Louisiana
LSU Health NEW ORLEANS
NIH
National Institute on Alcohol Abuse and Alcoholism
INTRODUCTION
Post Traumatic Stress Disorder (PTSD) affects around 7.7 million Americans every year.
PTSD diagnostic criteria includes persistent avoidance of distressing trauma-related stimuli after the event.
Corticotropin Releasing Factor (CRF) is a hormone that is critical for regulating emotions and is a key component of the stress pathway.
Previous research from our lab suggests that animals who displayed avoidance of a predator odor (bobcat urine, a natural stressor for rodents) show increases in extracellular CRF in the central nucleus of the amygdala (CeA).
CeL
CeM
CeC
BLA
HYPOTHESIS
We hypothesized that exogenous CRF administration bilaterally and site-specifically into the CeA would dose-dependently increase avoidance behavior in rats.
EXPERIMENTAL DESIGN
DAYS 1-7
DAYS 8-15
DAY 16
DAY 17
DAYS 18, 20, 22
DAYS 19, 21, 23
DAY 24
DAY 25
Acclimation (7 days)
Recovery (7 days)
3-chamber baseline
2-chamber baseline
Vehicle in Chamber A
CRF in Chamber B
CPA 2-Chamber Test
Euthanize rats/Histology
Cannula implantation
Male Wistar rats (n = 32) ~300-500 g.
Rats were counterbalanced for avoidance then assigned into 4 CRF dose groups (Vehicle [20% HBC pH=4.5], 0.05 µg CRF, 0.25 µg CRF, 0.5 µg CRF).
Infusions of 0.5 µL are injected over 2.5 min, then 1 min wait before removing injectors.
The Gilpin lab uses a model for testing individual differences in avoidance behavior using conditioned place avoidance (CPA).
Avoidance is defined as spending less time in a certain chamber post-conditioning.
RESULTS
Δ time in CRF chamber (s)
30
20
10
0
-10
-20
-30
0
0.05
0.25
0.5
CRF dose (ug)
Intra-CeA CRF administration appears to increase avoidance behavior in rats (p=0.051).
CONCLUSIONS
Preliminary data shows a nonsignificant trend that administering CRF into the CeA increases avoidance behavior in rats in a dose-dependent manner.
Similar to predator odor, CRF in the CeA produced avoidance.
These findings are part of our ongoing investigation of the effects of CRF in the CeA on anxiety-like behaviors.
REFERENCES
This research was supported by grant #R25AA021304 from the National Institute of Alcohol Abuse and Alcoholism at the National Institutes of Health.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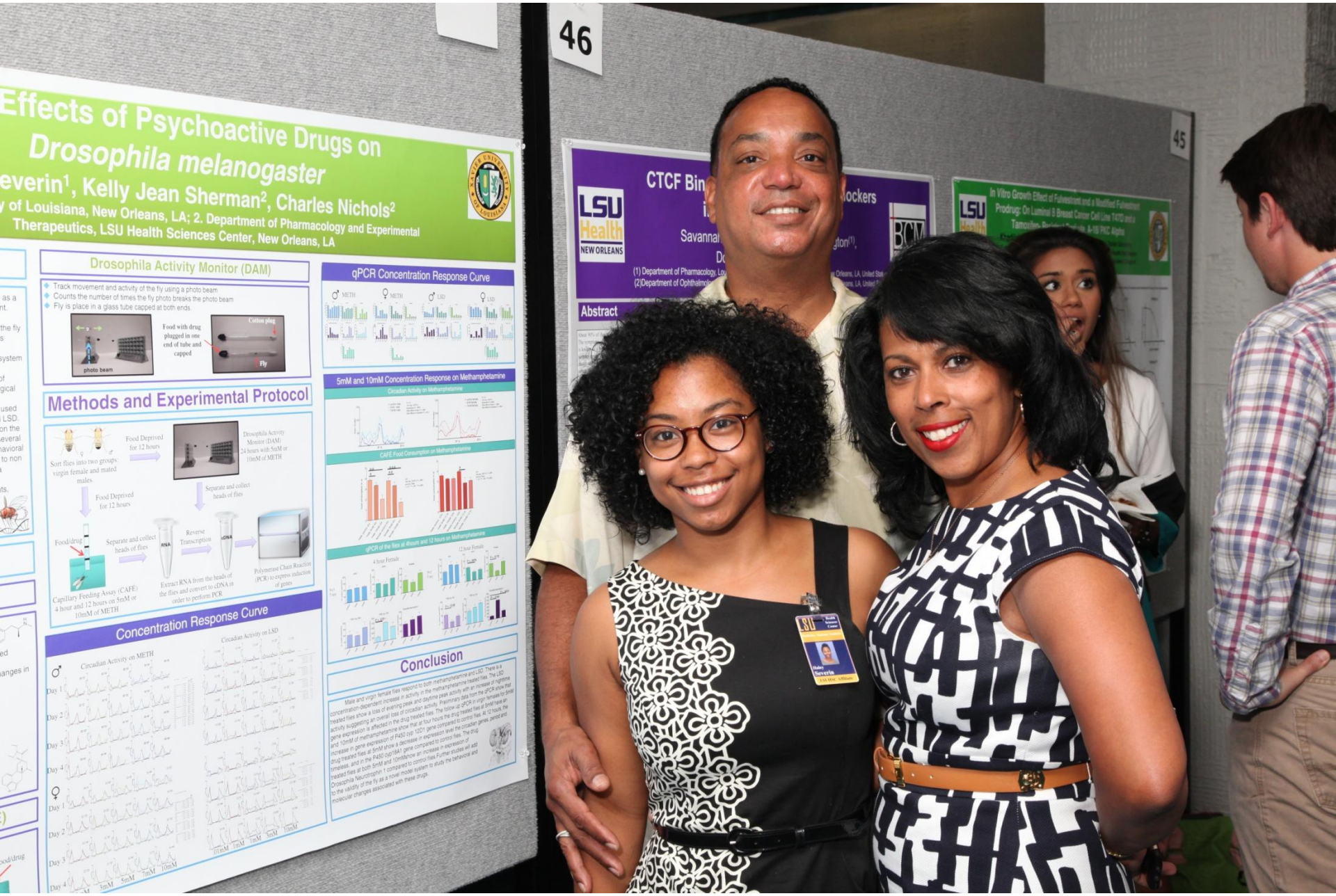
LSU Health NEW ORLEANS
The Effects of Psychoactive Drugs on Drosophila melanogaster
Haley Severin[1], Kelly Jean Sherman[2], Charles Nichols[2]
1. Xavier University of Louisiana, New Orleans, LA; 2. Department of Pharmacology and Experimental Therapeutics, LSU Health Sciences Center, New Orleans, LA
Introduction
Background
Psychoactive Drugs
Capillary Feeding Assay (CAFE)
Drosophila Activity Monitor (DAM)
Methods and Experimental Protocol
Concentration Response Curve
qPCR Concentration Response Curve
Conclusion
CTCF Binding Motifs as Enhancer
in Herpes Simplex Virus-
Savannah Sadaiappen, Shannan Washing
Donna M. Neumann, Ph.D(1)(2)
(1) Department of Pharmacology, Louisiana State University Health Sciences Center, New
(2)Department of Ophthalmology, Louisiana State University Health Sciences Center, New
Results


Effects of Psychoactive Drugs on
Drosophila melanogaster
everin[1], Kelly Jean Sherman[2], Charles Nichols[2]
y of Louisiana, New Orleans, LA; 2. Department of Pharmacology and Experimental
Therapeutics, LSU Health Sciences Center, New Orleans, LA
Drosophila Activity Monitor (DAM)
Track movement and activity of the fly using a photo beam
Counts the number of times the fly photo breaks the photo beam
Fly is place in a glass tube capped at both ends.
photo beam
Food with drug plugged in one end of tube and capped
Cotton plug
Fly
Methods and Experimental Protocol
Sort flies into two groups: virgin female and mated males.
Food Deprived for 12 hours
Drosophila Activity Monitor (DAM) 24 hours with 5mM or 10mM of METH
Food Deprived for 12 hours
Separate and collect heads of flies
Food/drug
Separate and collect heads of flies
RNA
Reverse Transcription
cDNA
Capillary Feeding Assay (CAFE) 4 hour and 12 hours on 5mM or 10mM of METH
Extract RNA from the heads of the flies and convert to cDNA in order to perform PCR
Polymerase Chain Reaction (PCR) to express induction of genes
Concentration Response Curve
Circadian Activity on METH
Circadian Activity on LSD
Day 1
Day 2
Day 3
Day 4
10mM 7mM 1mM 5mM 10mM
3mM 5mM 7mM 10mM
qPCR Concentration Response Curve
METH
METH
LSD
LSD
5mM and 10mM Concentration Response on Methamphetamine
Circadian Activity on Methamphetamine
CAFE Food Consumption on Methamphetamine
qPCR of the flies at 4hours and 12 hours on Methamphetamine
4 hour Female
12 hour Female
Conclusion
Male and virgin female flies respond to both methamphetamine and LSD. There is a concentration-dependent increase in activity in the methamphetamine treated flies. The LSD treated flies show a loss of evening peak and daytime peak activity with an increase of nighttime activity suggesting an overall loss of circadian activity. Preliminary data from the qPCR show that gene expression is affected in the drug treated flies. The follow up qPCR in virgin females for 5mM and 10mM of methamphetamine show that at four hours the drug treated flies at 5mM have an increase in gene expression of P450 cyp 12D1 gene compared to control flies. At 12 hours, the drug treated flies at 5mM show a decrease in expression level the circadian genes, period and timeless, and in the P450 cyp18A1 gene compared to control flies. The drug treated flies at both 5mM and 10mMshow an increase in expression of Drosophila Neurokinin 1 compared to control flies.Further studies will add to the validity of the fly as a novel model system to study the behavioral and molecular changes associated with these drugs.
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CTCF Bin
Abstract
BCM
In Vitro Growth Effect of Fulvestrant and a Modified Fulvestrant Prodrug: On Luminal B Breast Cancer Cell Line T47D and a Tamoxifen-Resistant Variant A-1/6 PKC Alpha

Chronic Binge Alcohol Administration Enhances Pre-Frontal Cortex Apoptotic Signaling In SIV-Infected Rhesus Macaques

Jacob Elnaggar, John Maxi, and Patricia Molina, M.D., Ph.D.
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NIH National Institute on Alcohol Abuse and Alcoholism

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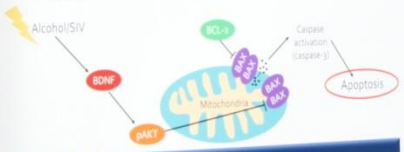
Introduction

- The frequency of heavy alcohol use in individuals living with HIV/AIDS is double that of the general population. Alcohol abuse increases the rate of HIV-associated neurocognitive disorder (HAND) development.
- In our non-human primate model of HIV infection, chronic binge alcohol (CBA) administration unmasks neurocognitive deficits in Simian Immunodeficiency Virus (SIV)-infected macaques.
- Neuronal apoptosis may be an underlying mechanism for neurocognitive disorders in CBA-administered SIV-infected macaques.
- **We hypothesized that there is an increase in pro-apoptotic signaling in the pre-frontal cortex of CBA/SIV infected macaques.**



Methods

- Male rhesus macaques were surgically fitted with a gastric catheter for the infusion of alcohol (13–14 g of ethanol/kg body weight per week; 30% w/v water) or sucrose starting 3 months prior to inoculation with SIVmac251.
- Three experimental groups: CBA/SIV (n=3), sucrose/SIV (n=3), and Naïve, SIV- (n=1)
- Animals were sacrificed at ~18 months after SIV
- Brains were excised and pre-frontal cortex isolated
- Protein expression of Bax, Bcl-2,and pAKT was determined by Western Blot. Data is normalized to beta-actin
- BDNF mRNA expression was determined by qPCR.

Anti-apoptosis

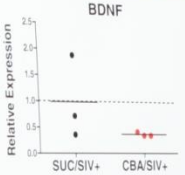


Figure 1. Relative mRNA expression of *Bdnf*. qPCR of pre-frontal cortex from sucrose/SIV+ and CBA/SIV+ animals. Dashed line represents the Naïve (SIV-) BDNF expression.

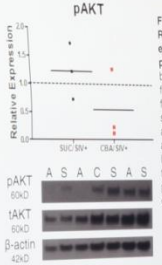


Figure 2. Relative protein expression of pAKT. Western blots of pre-frontal cortex from sucrose/SIV+ and CBA/SIV+ animals. Dashed line represents the Naïve (SIV-) pAKT expression.

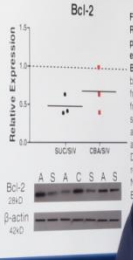
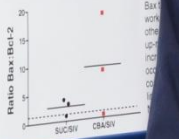


Figure 3. Relative protein expression of Bcl-2. Western blots of pre-frontal cortex from sucrose/SIV+ and CBA/SIV+ animals. Dashed line represent

Pro-apoptosis

Figure 4. Relative protein expression of Bax. Western blots of pre-frontal cortex from sucrose/SIV+ and CBA/SIV+ animals. Dashed line represents the Naïve (SIV-) Bax expression.



Summary

- Our results showed enhanced Bax expression and suppressed Bcl-2 expressio CBA/SIV+ macaques, when compared to the sucrose/SIV+ macaques.
- The ratio between Bax and Bcl-2 suggests enhanced apoptotic signaling in CB macaques.
- Decrease in pAKT and BDNF indicate less anti-apoptotic signaling in CBA/SIV
- These findings demonstrate the need for further investigation of the combinatio infection on neuronal apoptosis in animals as a potential mechanism underlying

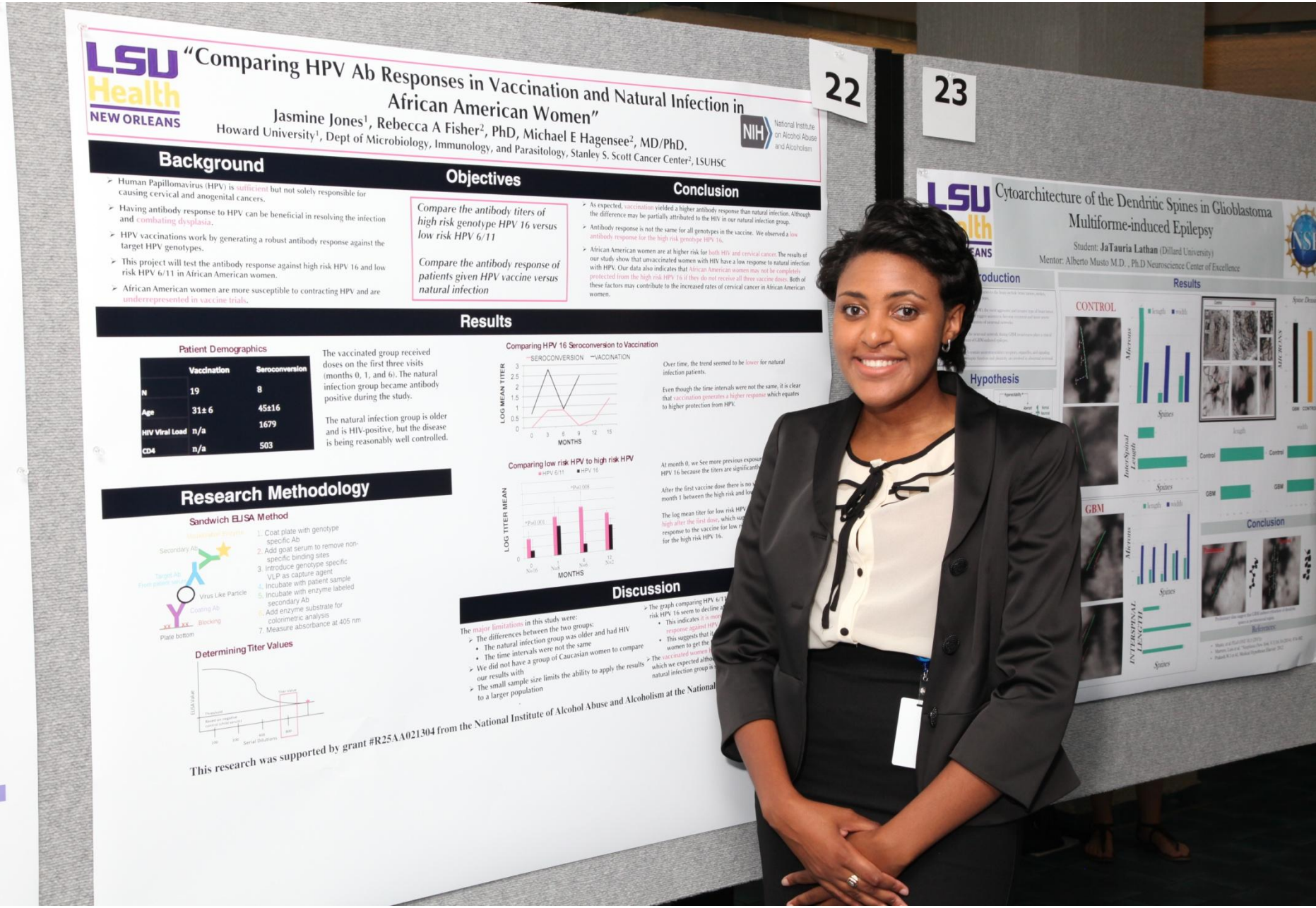
This research was supported by grant #R25AA021304 and AA09803 from the National Institute of Alcohol Abuse and Alcoholism at the National

Comparative Study of Accuracy:
of-Care vs Central Lab Urinalysis
an Urban Emergency Department
wards; Victoria Serven; Cara J. Joyce, PhD; L. Moreno-Walton, MD, MS
Department of Medicine, Section of Emergency Medicine, LSU-HSC, New Orleans

Most Impactful Categories

Results

Conclusions


LSU Health NEW ORLEANS
"Comparing HPV Ab Responses in Vaccination and Natural Infection in African American Women"
Jasmine Jones[1], Rebecca A Fisher[2], PhD, Michael E Hagensee[2], MD/PhD.
Howard University[1], Dept of Microbiology, Immunology, and Parasitology, Stanley S. Scott Cancer Center[2], LSUHSC
NIH National Institute on Alcohol Abuse and Alcoholism
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Background
Human Papillomavirus (HPV) is sufficient but not solely responsible for causing cervical and anogenital cancers.
Having antibody response to HPV can be beneficial in resolving the infection and combating dysplasia.
HPV vaccinations work by generating a robust antibody response against the target HPV genotypes.
This project will test the antibody response against high risk HPV 16 and low risk HPV 6/11 in African American women.
African American women are more susceptible to contracting HPV and are underrepresented in vaccine trials.
Objectives
Compare the antibody titers of high risk genotype HPV 16 versus low risk HPV 6/11
Compare the antibody response of patients given HPV vaccine versus natural infection
Conclusion
As expected, vaccination yielded a higher antibody response than natural infection. Although the difference may be partially attributed to the HIV in our natural infection group.
Antibody response is not the same for all genotypes in the vaccine. We observed a low antibody response for the high risk genotype HPV 16.
African American women are at higher risk for both HIV and cervical cancer. The results of our study show that unvaccinated women with HIV have a low response to natural infection with HPV. Our data also indicates that African American women may not be completely protected from the high risk HPV 16 if they do not receive all three vaccine doses. Both of these factors may contribute to the increased rates of cervical cancer in African American women.
Results
Patient Demographics
Vaccination | Seroconversion
N | 19 | 8
Age | 31± 6 | 45±16
HIV Viral Load | n/a | 1679
CD4 | n/a | 503
The vaccinated group received doses on the first three visits (months 0, 1, and 6). The natural infection group became antibody positive during the study.
The natural infection group is older and is HIV-positive, but the disease is being reasonably well controlled.
Comparing HPV 16 Seroconversion to Vaccination
SEROCONVERSION
VACCINATION
LOG MEAN TITER
MONTHS
Over time, the trend seemed to be lower for natural infection patients.
Even though the time intervals were not the same, it is clear that vaccination generates a higher response which equates to higher protection from HPV.
Comparing low risk HPV to high risk HPV
HPV 6/11
HPV 16
LOG TITER MEAN
MONTHS
Research Methodology
Sandwich ELISA Method
Secondary Ab
Target Ab
Virus Like Particle
Coating Ab
Blocking
Plate bottom
1. Coat plate with genotype specific Ab
2. Add goat serum to remove non-specific binding sites
3. Introduce genotype specific VLP as capture agent
4. Incubate with patient sample
5. Incubate with enzyme labeled secondary Ab
6. Add enzyme substrate for colorimetric analysis
7. Measure absorbance at 405 nm
Determining Titer Values
ELISA Value
Serial Dilutions
Discussion
The major limitations in this study were:
The differences between the two groups:
The natural infection group was older and had HIV
The time intervals were not the same
We did not have a group of Caucasian women to compare our results with
The small sample size limits the ability to apply the results to a larger population
This research was supported by grant #R25AA021304 from the National Institute of Alcohol Abuse and Alcoholism at the National
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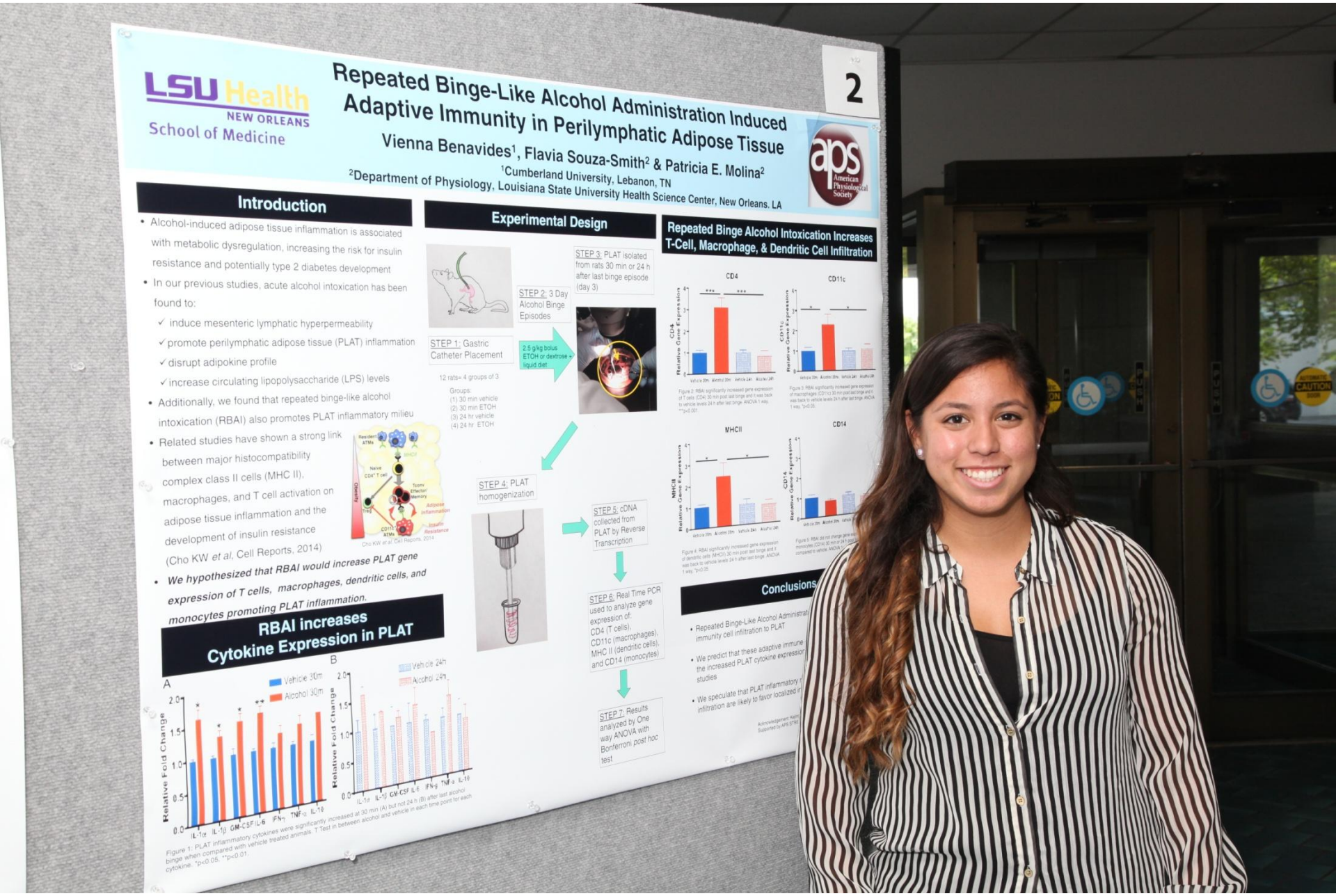
Cytoarchitecture of the Dendritic Spines in Glioblastoma Multiforme-induced Epilepsy
Student: JaTauria Lathan (Dillard University)
Mentor: Alberto Musto M.D. , Ph.D Neuroscience Center of Excellence
Hypothesis
Results
CONTROL
GBM
Conclusion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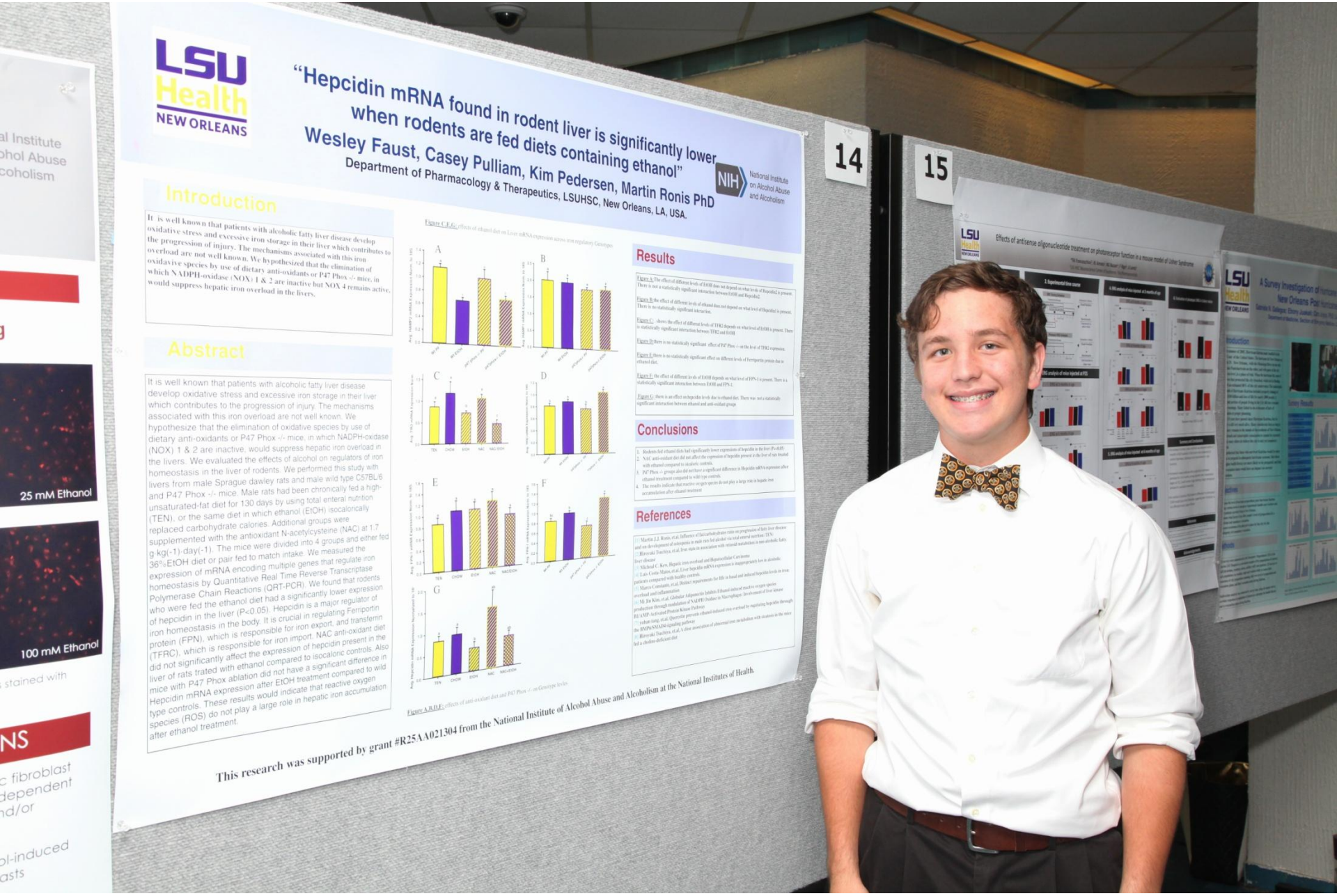
Repeated Binge-Like Alcohol Administration Induced Adaptive Immunity in Perilymphatic Adipose Tissue
Vienna Benavides[1], Flavia Souza-Smith[2] & Patricia E. Molina[2]
[1]Cumberland University, Lebanon, TN
[2]Department of Physiology, Louisiana State University Health Science Center, New Orleans, LA
aps American Physiological Society
Introduction
• Alcohol-induced adipose tissue inflammation is associated with metabolic dysregulation, increasing the risk for insulin resistance and potentially type 2 diabetes development
• In our previous studies, acute alcohol intoxication has been found to:
✓ induce mesenteric lymphatic hyperpermeability
✓ promote perilymphatic adipose tissue (PLAT) inflammation
✓ disrupt adipokine profile
✓ increase circulating lipopolysaccharide (LPS) levels
• Additionally, we found that repeated binge-like alcohol intoxication (RBAI) also promotes PLAT inflammatory milieu
• Related studies have shown a strong link between major histocompatibility complex class II cells (MHC II), macrophages, and T cell activation on adipose tissue inflammation and the development of insulin resistance (Cho KW et al, Cell Reports, 2014)
Cho KW et al, Cell Reports, 2014
• We hypothesized that RBAI would increase PLAT gene expression of T cells, macrophages, dendritic cells, and monocytes promoting PLAT inflammation.
Experimental Design
STEP 1: Gastric Catheter Placement
STEP 2: 3 Day Alcohol Binge Episodes
2.5 g/kg bolus ETOH or dextrose + liquid diet
STEP 3: PLAT isolated from rats 30 min or 24 h after last binge episode (day 3)
12 rats= 4 groups of 3
Groups:
(1) 30 min vehicle
(2) 30 min ETOH
(3) 24 hr vehicle
(4) 24 hr ETOH
STEP 4: PLAT homogenization
STEP 5: cDNA collected from PLAT by Reverse Transcription
STEP 6: Real Time PCR used to analyze gene expression of: CD4 (T cells), CD11c (macrophages), MHC II (dendritic cells), and CD14 (monocytes)
STEP 7: Results analyzed by One way ANOVA with Bonferroni post hoc test
RBAI increases Cytokine Expression in PLAT
A
B
Vehicle 30m
Alcohol 30m
Vehicle 24h
Alcohol 24h
Relative Fold Change
IL-1α IL-1β GM-CSF IL-6 IFN-γ TNF-α IL-10
Figure 1: PLAT inflammatory cytokines were significantly increased at 30 min (A) but not 24 h (B) after last alcohol binge when compared with vehicle treated animals. T Test in between alcohol and vehicle in each time point for each cytokine. *p<0.05, **p<0.01.
Repeated Binge Alcohol Intoxication Increases T-Cell, Macrophage, & Dendritic Cell Infiltration
CD4
CD11c
MHCII
CD14
Relative Gene Expression
Conclusions
• Repeated Binge-Like Alcohol Administrati
immunity cell infiltration to PLAT
• We predict that these adaptive immune
the increased PLAT cytokine expression
studies
• We speculate that PLAT inflammatory
infiltration are likely to favor localized


LSU Health NEW ORLEANS
"Hepcidin mRNA found in rodent liver is significantly lower when rodents are fed diets containing ethanol"
Wesley Faust, Casey Pulliam, Kim Pedersen, Martin Ronis PhD
Department of Pharmacology & Therapeutics, LSUHSC, New Orleans, LA, USA.
NIH National Institute on Alcohol Abuse and Alcoholism
Introduction
It is well known that patients with alcoholic fatty liver disease develop oxidative stress and excessive iron storage in their liver which contributes to the progression of injury. The mechanisms associated with this iron overload are not well known. We hypothesized that the elimination of oxidative species by use of dietary anti-oxidants or P47 Phox -/- mice, in which NADPH-oxidase (NOX) 1 & 2 are inactive but NOX 4 remains active, would suppress hepatic iron overload in the livers.
Abstract
It is well known that patients with alcoholic fatty liver disease develop oxidative stress and excessive iron storage in their liver which contributes to the progression of injury. The mechanisms associated with this iron overload are not well known. We hypothesize that the elimination of oxidative species by use of dietary anti-oxidants or P47 Phox -/- mice, in which NADPH-oxidase (NOX) 1 & 2 are inactive, would suppress hepatic iron overload in the livers. We evaluated the effects of alcohol on regulators of iron homeostasis in the liver of rodents. We performed this study with livers from male Sprague dawley rats and male wild type C57BL/6 and P47 Phox -/- mice. Male rats had been chronically fed a high-unsaturated-fat diet for 130 days by using total enteral nutrition (TEN), or the same diet in which ethanol (EtOH) isocalorically replaced carbohydrate calories. Additional groups were supplemented with the antioxidant N-acetylcysteine (NAC) at 1.7 g·kg(-1)·day(-1). The mice were divided into 4 groups and either fed 36%EtOH diet or pair fed to match intake. We measured the expression of mRNA encoding multiple genes that regulate iron homeostasis by Quantitative Real Time Reverse Transcriptase Polymerase Chain Reactions (QRT-PCR). We found that rodents who were fed the ethanol diet had a significantly lower expression of hepcidin in the liver (P<0.05). Hepcidin is a major regulator of iron homeostasis in the body. It is crucial in regulating Ferriportin protein (FPN), which is responsible for iron export, and transferrin (TFRC), which is responsible for iron import. NAC anti-oxidant diet did not significantly affect the expression of hepcidin present in the liver of rats treated with ethanol compared to isocaloric controls. Also mice with P47 Phox ablation did not have a significant difference in Hepcidin mRNA expression after EtOH treatment compared to wild type controls. These results would indicate that reactive oxygen species (ROS) do not play a large role in hepatic iron accumulation after ethanol treatment.
Figure C,E,G: effects of ethanol diet on Liver mRNA expression across iron regulatory Genotypes
Figure A,B,D,F: effects of anti-oxidant diet and P47 Phox -/- on Genotype levles
Results
Figure A: The effect of different levels of EtOH does not depend on what levels of Hepcidin2 is present. There is not a statistically significant interaction between EtOH and Hepcidin2.
Figure B:the effect of different levels of ethanol does not depend on what level of Hepcidin1 is present. There is no statistically significant interaction.
Figure C: -shows the effect of different levels of TFR2 depends on what level of EtOH is present. There is statistically significant interaction between TFR2 and EtOH
Figure D:there is no statistically significant effect of P47 Phox -/- on the level of TFR2 expression.
Figure E:there is no statistically significant effect on different levels of Ferriportin protein due to ethanol diet.
Figure F: the effect of different levels of EtOH depends on what level of FPN-1 is present. There is a statistically significant interaction between EtOH and FPN-1.
Figure G: there is an effect on hepcidin levels due to ethanol diet. There was not a statistically significant interaction between ethanol and anti-oxidant groups
Conclusions
1. Rodents fed ethanol diets had significantly lower expressions of hepcidin in the liver (P<0.05)
2. NAC anti-oxidant diet did not affect the expression of hepcidin present in the liver of rats treated with ethanol compared to isocaloric controls.
3. P47 Phox -/- groups also did not have a significant difference in Hepcidin mRNA expression after ethanol treatment compared to wild type controls.
4. The results indicate that reactive oxygen species do not play a large role in hepatic iron accumulation after ethanol treatment
References
This research was supported by grant #R25AA021304 from the National Institute of Alcohol Abuse and Alcoholism at the National Institutes of Health.
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Effects of antisense oligonucleotide treatment on photoreceptor function in a mouse model of Usher Syndrome
A Survey Investigation of Homeopathy in New Orleans Poor Homeopathy
25 mM Ethanol
100 mM Ethanol
stained with
NS
c fibroblast
dependent
nd/or
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coholism


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Binge Alcohol Administration Enhances
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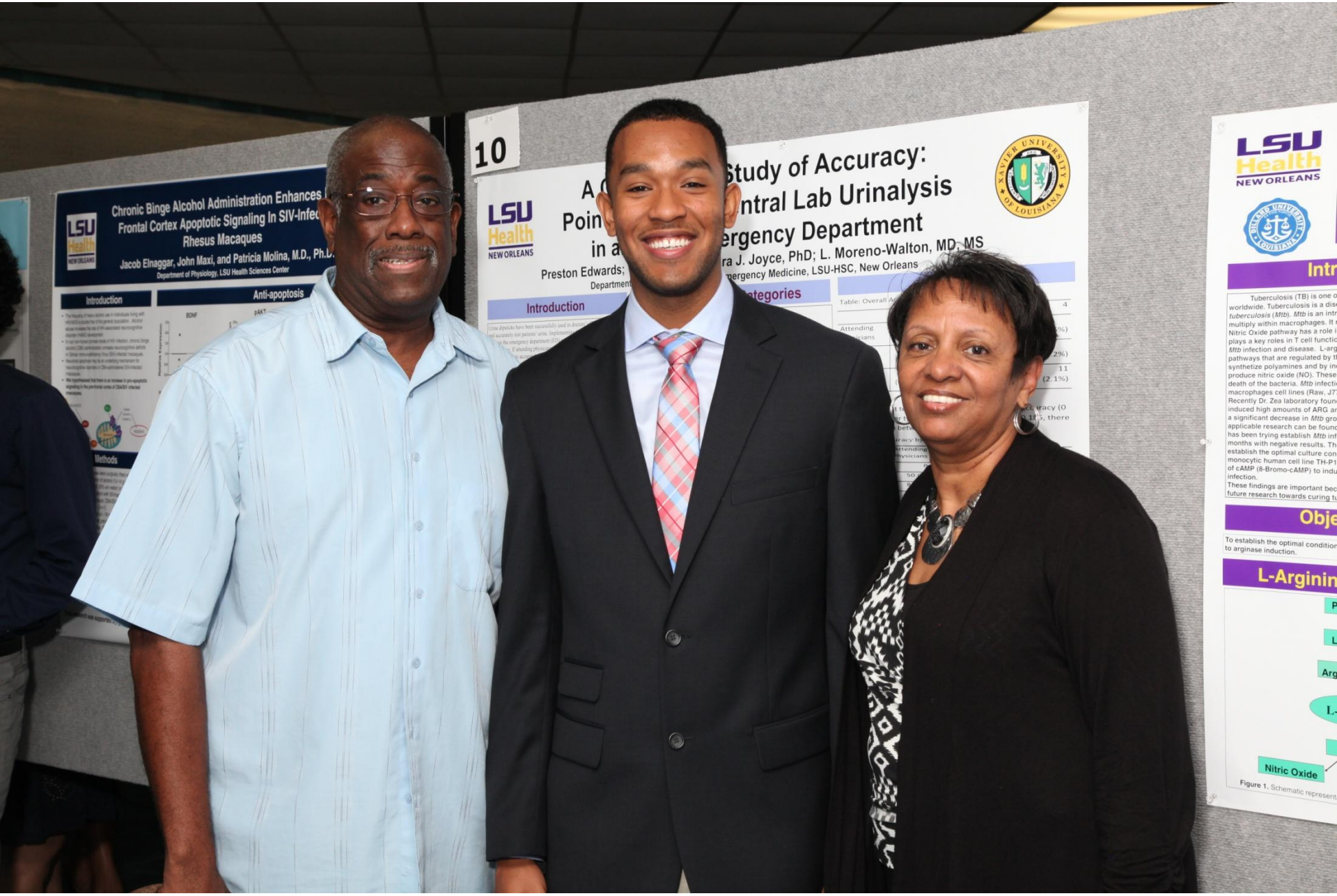
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LSU Health NEW ORLEANS
Chronic Binge Alcohol Administration Enhances
Frontal Cortex Apoptotic Signaling In SIV-Infec
Rhesus Macaques
Jacob Elnaggar, John Maxi, and Patricia Molina, M.D., Ph.D.
Department of Physiology, LSU Health Sciences Center
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Anti-apoptosis
Methods
Study of Accuracy:
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ergency Department
Preston Edwards;
ra J. Joyce, PhD; L. Moreno-Walton, MD, MS
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LSU Health NEW ORLEANS
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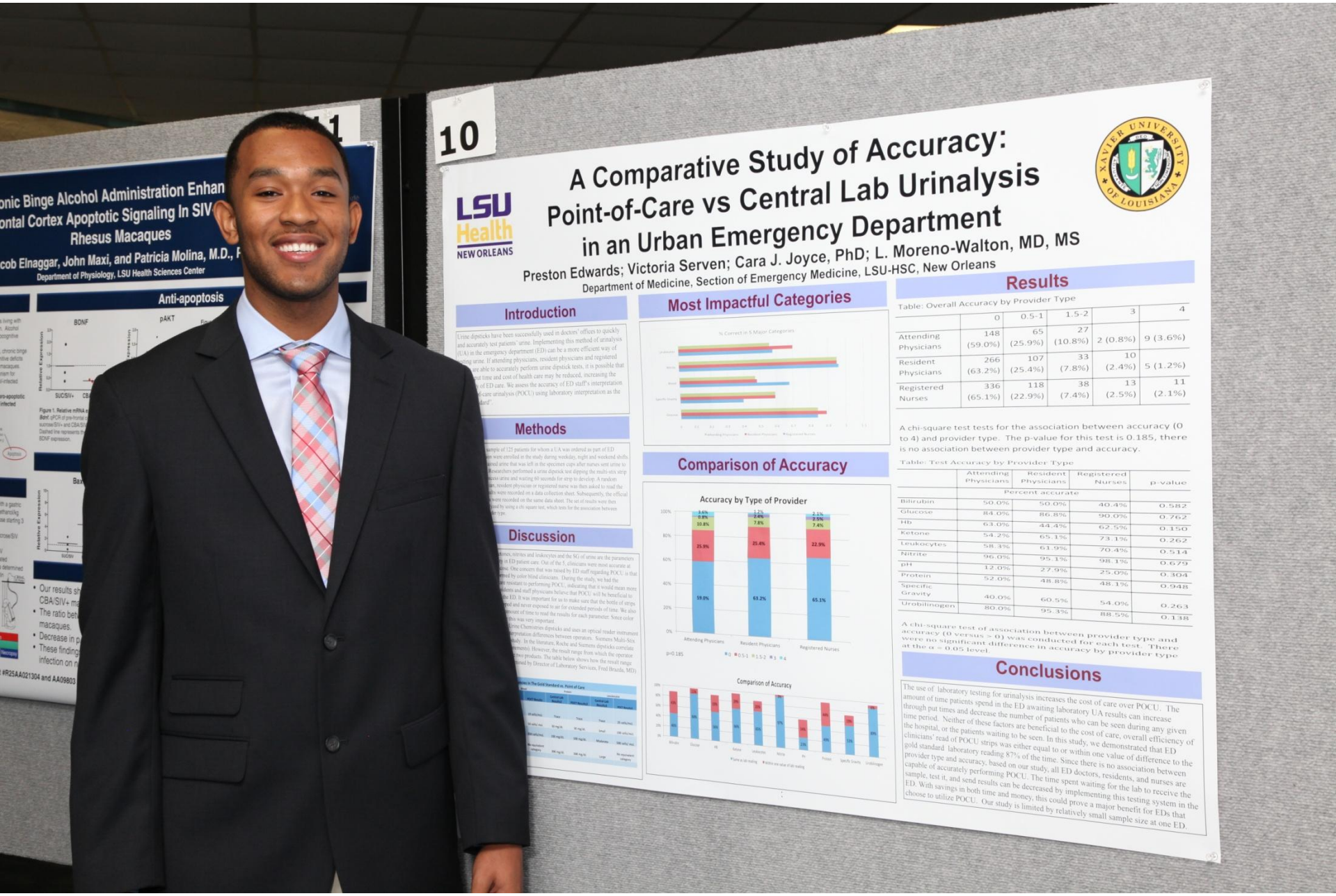
Nitric Oxide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A Comparative Study of Accuracy: Point-of-Care vs Central Lab Urinalysis in an Urban Emergency Department
LSU Health NEW ORLEANS
XAVIER UNIVERSITY OF LOUISIANA
Preston Edwards; Victoria Serven; Cara J. Joyce, PhD; L. Moreno-Walton, MD, MS
Department of Medicine, Section of Emergency Medicine, LSU-HSC, New Orleans
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Comparison of Accuracy
Accuracy by Type of Provider
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Table: Overall Accuracy by Provider Type
Table: Test Accuracy by Provider Type
Conclusions
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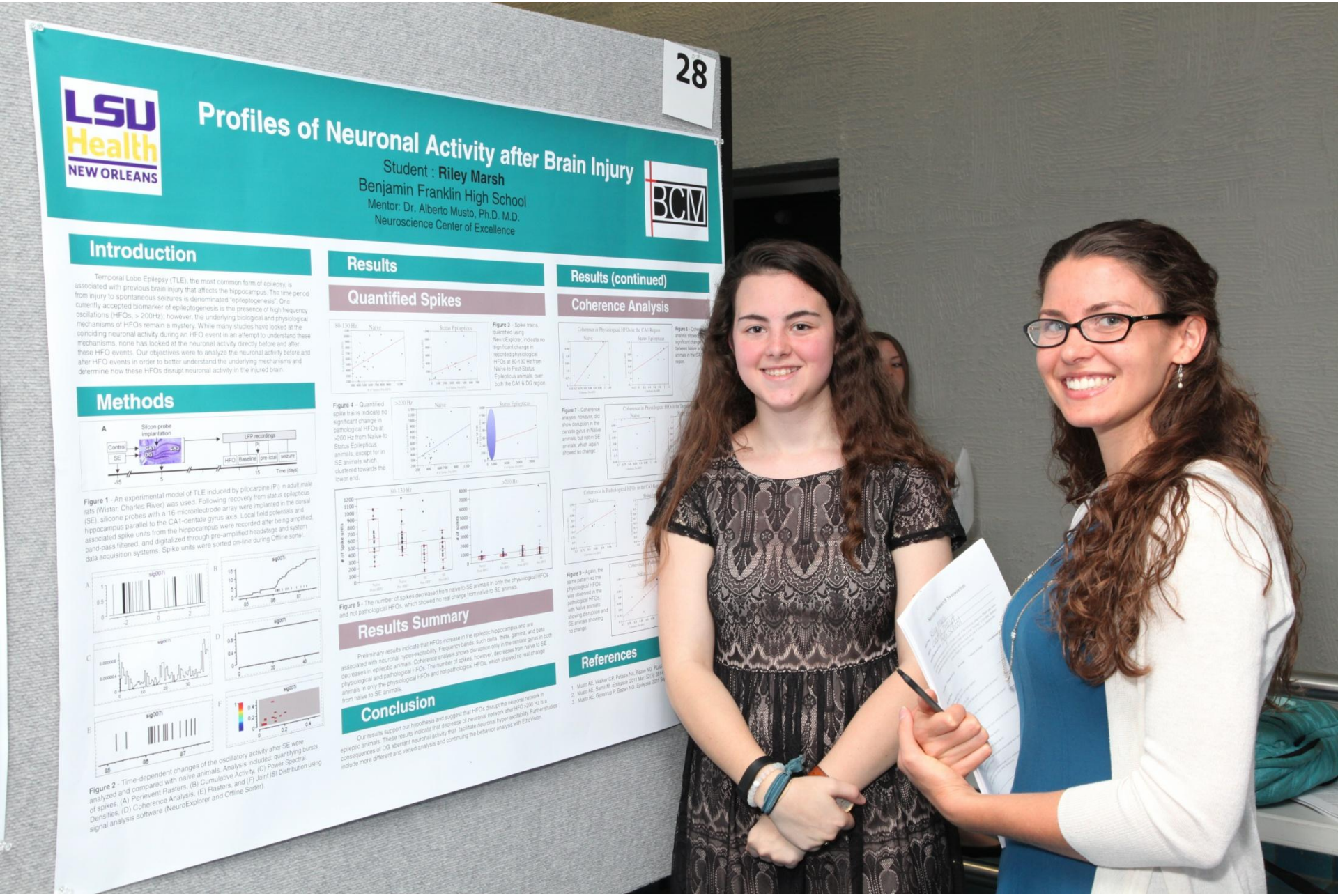
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LSU Health NEW ORLEANS
Profiles of Neuronal Activity after Brain Injury
Student : Riley Marsh
Benjamin Franklin High School
Mentor: Dr. Alberto Musto, Ph.D. M.D.
Neuroscience Center of Excellence
BCM
Introduction
Methods
Results
Quantified Spikes
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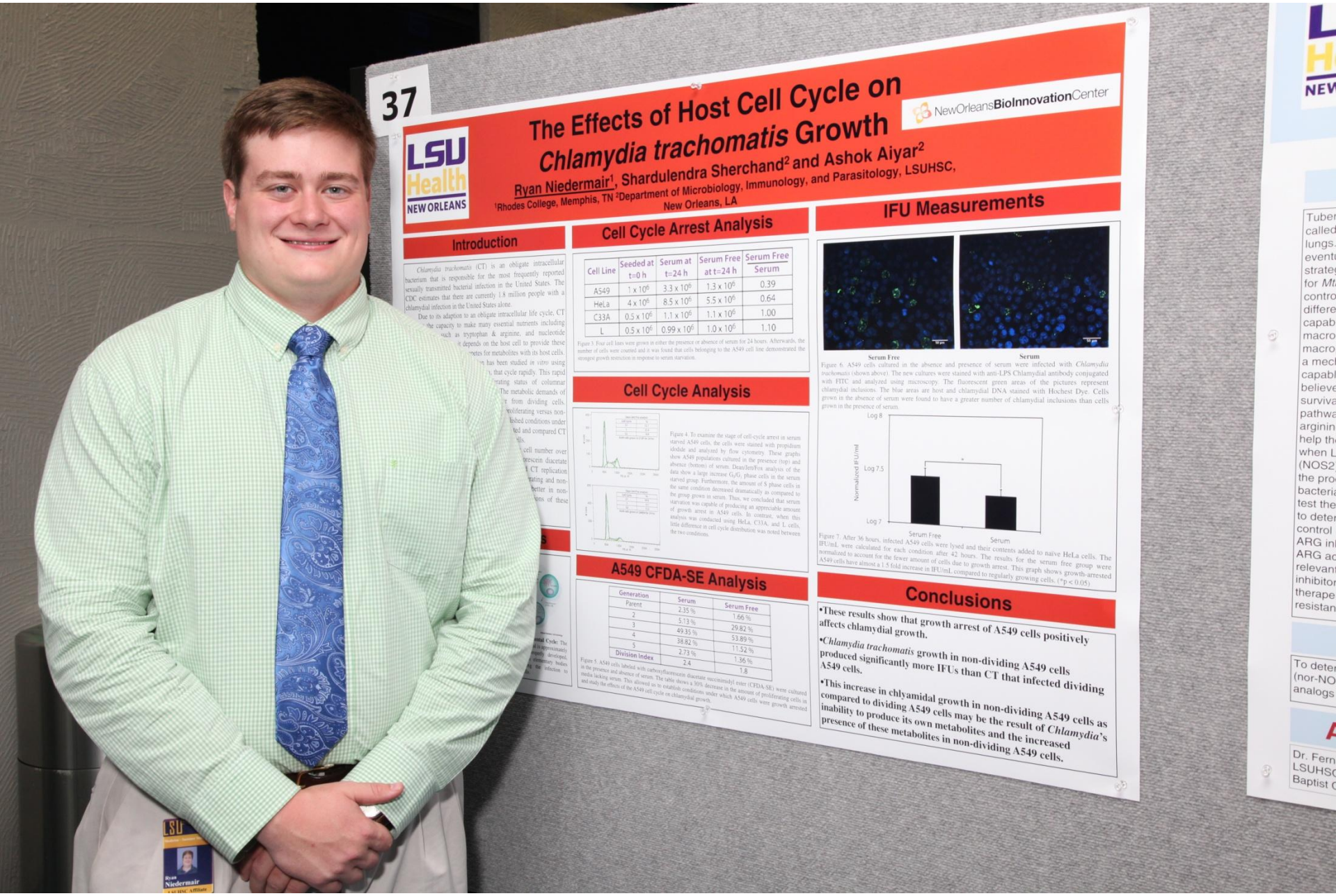
Conclusion
Results (continued)
Coherence Analysis
References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LSU Health NEW ORLEANS
NewOrleansBioInnovationCenter
The Effects of Host Cell Cycle on Chlamydia trachomatis Growth
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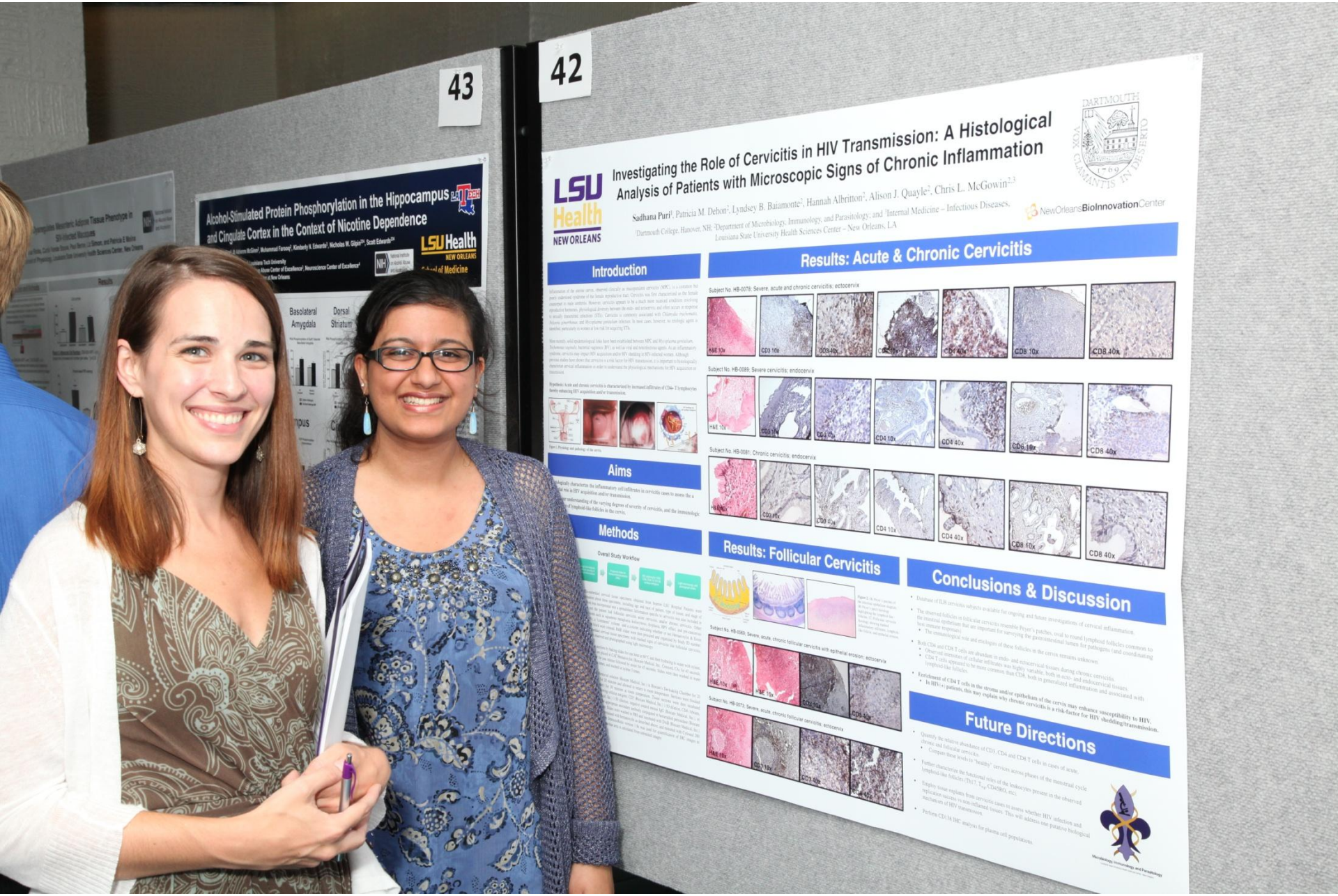
[1]Rhodes College, Memphis, TN [2]Department of Microbiology, Immunology, and Parasitology, LSUHSC, New Orleans, LA
Introduction
Cell Cycle Arrest Analysis
Cell Line | Seeded at t=0 h | Serum at t=24 h | Serum Free at t=24 h | Serum Free / Serum
A549 | 1 x 10^6 | 3.3 x 10^6 | 1.3 x 10^6 | 0.39
HeLa | 4 x 10^6 | 8.5 x 10^6 | 5.5 x 10^6 | 0.64
C33A | 0.5 x 10^6 | 1.1 x 10^6 | 1.1 x 10^6 | 1.00
L | 0.5 x 10^6 | 0.99 x 10^6 | 1.0 x 10^6 | 1.10
Figure 3. Four cell lines were grown in either the presence or absence of serum for 24 hours. Afterwards, the number of cells were counted and it was found that cells belonging to the A549 cell line demonstrated the strongest growth restriction in response to serum starvation.
Cell Cycle Analysis
Figure 4. To examine the stage of cell-cycle arrest in serum starved A549 cells, the cells were stained with propidium iodide and analyzed by flow cytometry. These graphs show A549 populations cultured in the presence (top) and absence (bottom) of serum. Dean/Jett/Fox analysis of the data show a large increase G_0/G_1 phase cells in the serum starved group. Furthermore, the amount of S phase cells in the same condition decreased dramatically as compared to the group grown in serum. Thus, we concluded that serum starvation was capable of producing an appreciable amount of growth arrest in A549 cells. In contrast, when this analysis was conducted using HeLa, C33A, and L cells, little difference in cell cycle distribution was noted between the two conditions.
A549 CFDA-SE Analysis
Generation | Serum | Serum Free
Parent | 2.35 % | 1.66 %
2 | 5.13 % | 29.82 %
3 | 49.35 % | 53.89 %
4 | 38.82 % | 11.52 %
5 | 2.73 % | 1.36 %
Division Index | 2.4 | 1.8
Figure 5. A549 cells labeled with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) were cultured in the presence and absence of serum. The table shows a 30% decrease in the amount of proliferating cells in media lacking serum. This allowed us to establish conditions under which A549 cells were growth arrested and study the effects of the A549 cell cycle on chlamydial growth.
IFU Measurements
Serum Free
Serum
Figure 6. A549 cells cultured in the absence and presence of serum were infected with Chlamydia trachomatis (shown above). The new cultures were stained with anti-LPS Chlamydial antibody conjugated with FITC and analyzed using microscopy. The fluorescent green areas of the pictures represent chlamydial inclusions. The blue areas are host and chlamydial DNA stained with Hochest Dye. Cells grown in the absence of serum were found to have a greater number of chlamydial inclusions than cells grown in the presence of serum.
Log 8
Log 7.5
Log 7
Normalized IFU/ml
Serum Free
Serum
Figure 7. After 36 hours, infected A549 cells were lysed and their contents added to naive HeLa cells. The IFU/mL were calculated for each condition after 42 hours. The results for the serum free group were normalized to account for the fewer amount of cells due to growth arrest. This graph shows growth-arrested A549 cells have almost a 1.5 fold increase in IFU/mL compared to regularly growing cells. (*p < 0.05)
Conclusions
•These results show that growth arrest of A549 cells positively affects chlamydial growth.
•Chlamydia trachomatis growth in non-dividing A549 cells produced significantly more IFUs than CT that infected dividing A549 cells.
•This increase in chlyamidal growth in non-dividing A549 cells as compared to dividing A549 cells may be the result of Chlamydia's inability to produce its own metabolites and the increased presence of these metabolites in non-dividing A549 cells.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Alcohol-Stimulated Protein Phosphorylation in the Hippocampus and Cingulate Cortex in the Context of Nicotine Dependence
LSU Health NEW ORLEANS School of Medicine
Basolateral Amygdala
Dorsal Striatum
LSU Health NEW ORLEANS
Investigating the Role of Cervicitis in HIV Transmission: A Histological Analysis of Patients with Microscopic Signs of Chronic Inflammation
Sadhana Puri[1], Patricia M. Dehon[2], Lyndsey B. Baiamonte[2], Hannah Albritton[2], Alison J. Quayle[2], Chris L. McGowin[2,3]
[1]Dartmouth College, Hanover, NH; [2]Department of Microbiology, Immunology, and Parasitology; and [3]Internal Medicine – Infectious Diseases, Louisiana State University Health Sciences Center – New Orleans, LA
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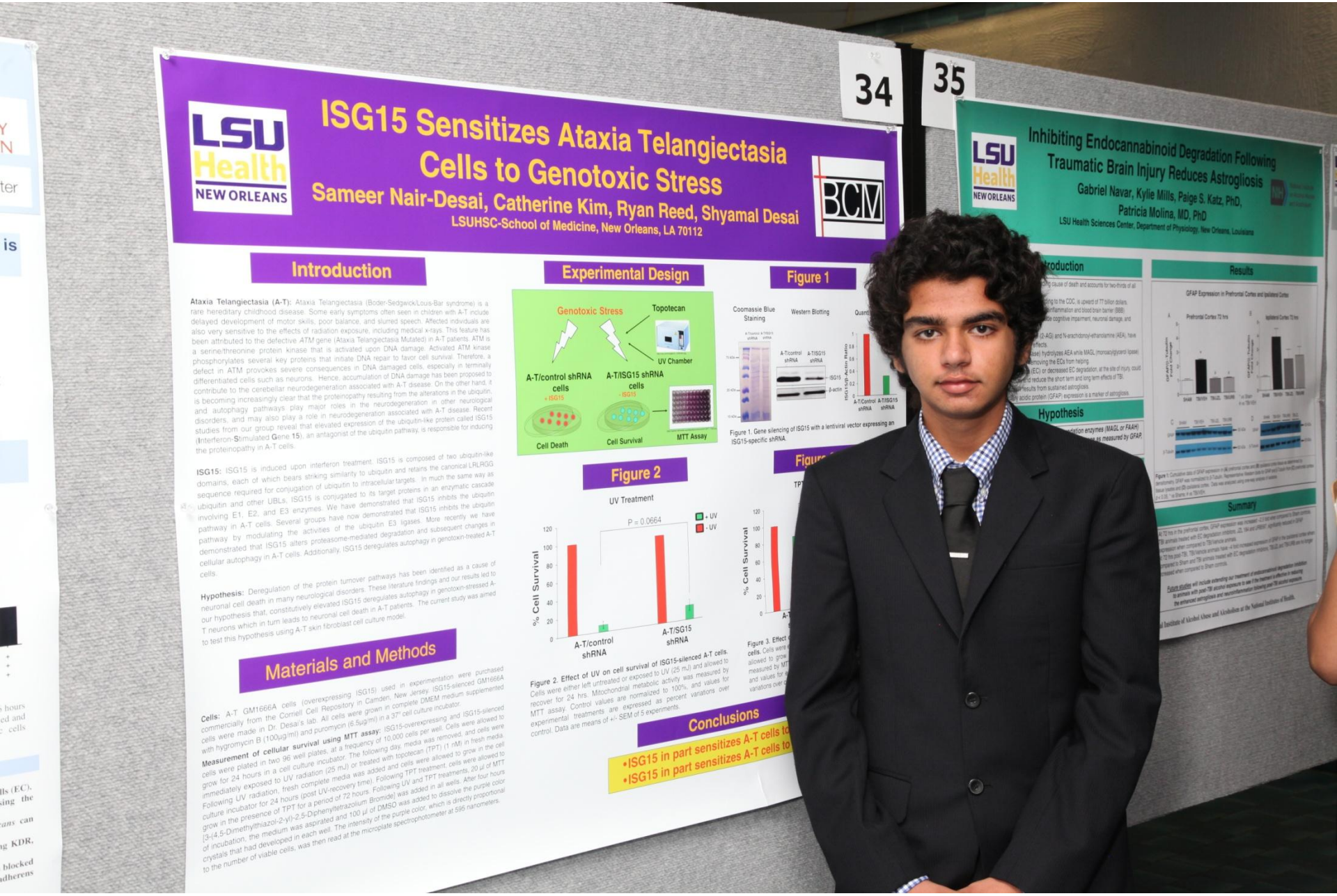
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LSU Health NEW ORLEANS
ISG15 Sensitizes Ataxia Telangiectasia Cells to Genotoxic Stress
Sameer Nair-Desai, Catherine Kim, Ryan Reed, Shyamal Desai
LSUHSC-School of Medicine, New Orleans, LA 70112
BCM
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UV Chamber
A-T/control shRNA cells
A-T/ISG15 shRNA cells
Cell Death
Cell Survival
MTT Assay
UV Treatment
P = 0.0664
% Cell Survival
A-T/control shRNA
A-T/SG15 shRNA
Inhibiting Endocannabinoid Degradation Following Traumatic Brain Injury Reduces Astrogliosis
Gabriel Navar, Kylie Mills, Paige S. Katz, PhD, Patricia Molina, MD, PhD
LSU Health Sciences Center, Department of Physiology, New Orleans, Louisiana
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CTCF Binding Motifs as Enhancer Blockers in Herpes Simplex Virus-1

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(2)Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA, United States

Abstract

About 90% of the adult human population carries Herpes Simplex Virus- Type 1. The symptoms are not always obvious, since the virus has the ability to enter what is known as a latent (dormant) cycle. During this latent period, HSV-1 is located in sensory neurons of the trigeminal ganglia, a system of neurons that connects oral, nasal, and ocular regions. Therefore, an HSV-1 mouth infection has potential to cause devastating ocular lesions. The lone fragment of the HSV-1 genome is transcribed into mRNA which becomes the Latency Associated Transcript (LAT) during latency. Lytic genes are repressed during latency, which the virus establishes through epigenetic mechanisms. In the latent cycle, the LAT exists as euchromatin, while lytic genes are tightly repressed and bundled into bodies, forming heterochromatin. Seven distinct CTCF binding motifs are found in the HSV-1 genome and are positioned in an orientation that flanks the LAT and each (Immediate Early) IE region separately. In the literature, it is established that these euchromatin segments, but this mechanism has not yet been studied in HSV-1. One possibility in the HSV-1 context is that CTCF motifs act as insulators that are enhancer-blockers, they may repress the expression of distal genes activated by an enhancer. Through this mechanism, the transcription of the LAT could be monopolized ensuring that the lytic genes are not expressed. Our goal in this study was to generate reporter-construct for two CTCF binding motifs in HSV-1, known as B4 and B5, which would subsequently be used to determine luciferase expression in transient transfection assays.

Background Information

A hallmark trait of HSV-1 is its ability to establish latency in the sensory neurons in the trigeminal ganglia. About 90% of the adult human population carries Herpes Simplex Virus-Type 1. Symptoms of infection are not always apparent, since the virus establishes latency. In the context of HSV-1, there have been seven identified CTCF binding domains in the genome. The location of these binding domains is interesting, they flank the LAT regions and each of the immediate early gene regions. During latency, each of these gene regions binds the cellular insulator protein CTCF. Based on these reports, the binding of the insulator protein CTCF is fundamental to the maintenance of latency and that loss of CTCF binding plays a role in the transition from latency to reactivation.

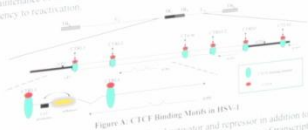

Figure A: CTCF Binding Motifs in HSV-1

CTCF can function as both a transcriptional activator and repressor in addition to acting as an insulator protein. CTCF has the ability to act as a co-regulator of transcription on genes adjacent to where it binds. CTCF can also bind to nuclear structural proteins, histone modifying enzymes and to other CTCF molecules. The binding of CTCF elicits a multitude of functions, including enhancer blocking, gene activation or gene repression in a linear manner. When CTCF binds to one of these motifs it has these abilities. The binding can be used to repress the lytic genes necessary for reactivation.

Results

Synthesis of CV+LTE Construct

Figure 1: Gel electrophoresis of LAT enhancer PCR products using 17syn+.

Figure 2: Gel electrophoresis of restriction enzyme digested CV and the LAT enhancer. Digested with restriction enzymes Nhe1 and Mlu1.

Figure 3: Colonies of transformed *E.coli* containing the CV and LTE ligated together.

CV and B4 PCR and Restriction Digestion

Figure 4: Gel electrophoresis of B4 PCR products using 17syn+.

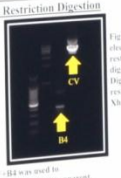

Figure 5: Gel electrophoresis of restriction enzyme digested CV and B4. Digested with restriction enzymes Xho1 and Nhe1.

*When ligated CV+B4 was used to transform *E.coli*, no colonies were apparent, it did not contain the construct.

B5 PCR

Figure 6: Gel electrophoresis of B5 PCR products using 17syn+.

Control Vector

Amplified regions of the constructs, each containing specific segments of HSV-1 were ligated to the control vector.

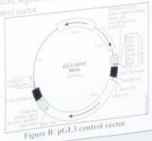

Figure B: pGL3 control vector

Methods

Synthesis of Constructs:

Constructs, each containing specific segments of HSV-1 DNA (17 syn+). Amplified regions were ligated to the commercially available pGL3 control vector(CV) (Figure B).

The following constructs were generated:

- CV
- CV+LTE
- LTE
- B4
- CV+B4
- B5

Techniques used to synthesize products:

- **Polymerase Chain Reaction (PCR)**
  Each segment of the HSV-1 17syn+ DNA was amplified using primers specific to the region of interest. The following primer sites were:
  LTE (589 bp): Mlu1 (118888), Nhe1(119477)
  B4 (400 bp): Nhe1(126376), Xho1(126758)
  B5 (1777 bp): Nhe1(128750), Xho1(128527)
- **Restriction Digestion**
  Sticky ends were created on the pGL3 control vector as well as the insert for each construct. The same restriction enzymes were used for the vector and insert for each construct. This insured that the two could be ligated.
- **Gel Electrophoresis**
  The restriction-digested vector and insert (for each construct) were purified by gel through a 1% agarose gel.
- **Gel DNA Recovery**
  The Zymoclean™ Gel DNA Recovery Kit was used to obtain the restriction DNA samples (both vector and insert) from the 1% agarose gel.
- **Ligation**
  The vector and insert were joined. This was accomplished by Quick Ligation solution of Quick Ligase Buffer.
- **Bacterial (*E. coli*) transformation**
  DH5 alpha competent *E. coli* cells were transformed with each construct. This allows us to produce the constructs in bulk. Some *E. coli* cells were transformed without insert to serve as a negative control.

Plating

Each tube of transformed *E. coli* was spread on plates containing LB ampicillin. Selected clones were then grown in 5 mL Terrific Broth with amp

- **Bacterial DNA Extraction**
  The Zyppy™ Plasmid Miniprep Kit was used to extract the DNA from each *E. coli* grown in broth. The DNA samples were then restriction digested and 1% agarose gel. This allowed us to determine which *E. coli* samples contained correctly ligated constructs.

Future Work

After ligation the plasmid DNA will be used to transform competent *E. coli*. Colonies will be selected and their DNA will be extracted. During this the host bacteria cells will be lysed and the proteins will be separated. The DNA will then be restriction digested again and verified by gel analysis insert is present in the construct.


LSU
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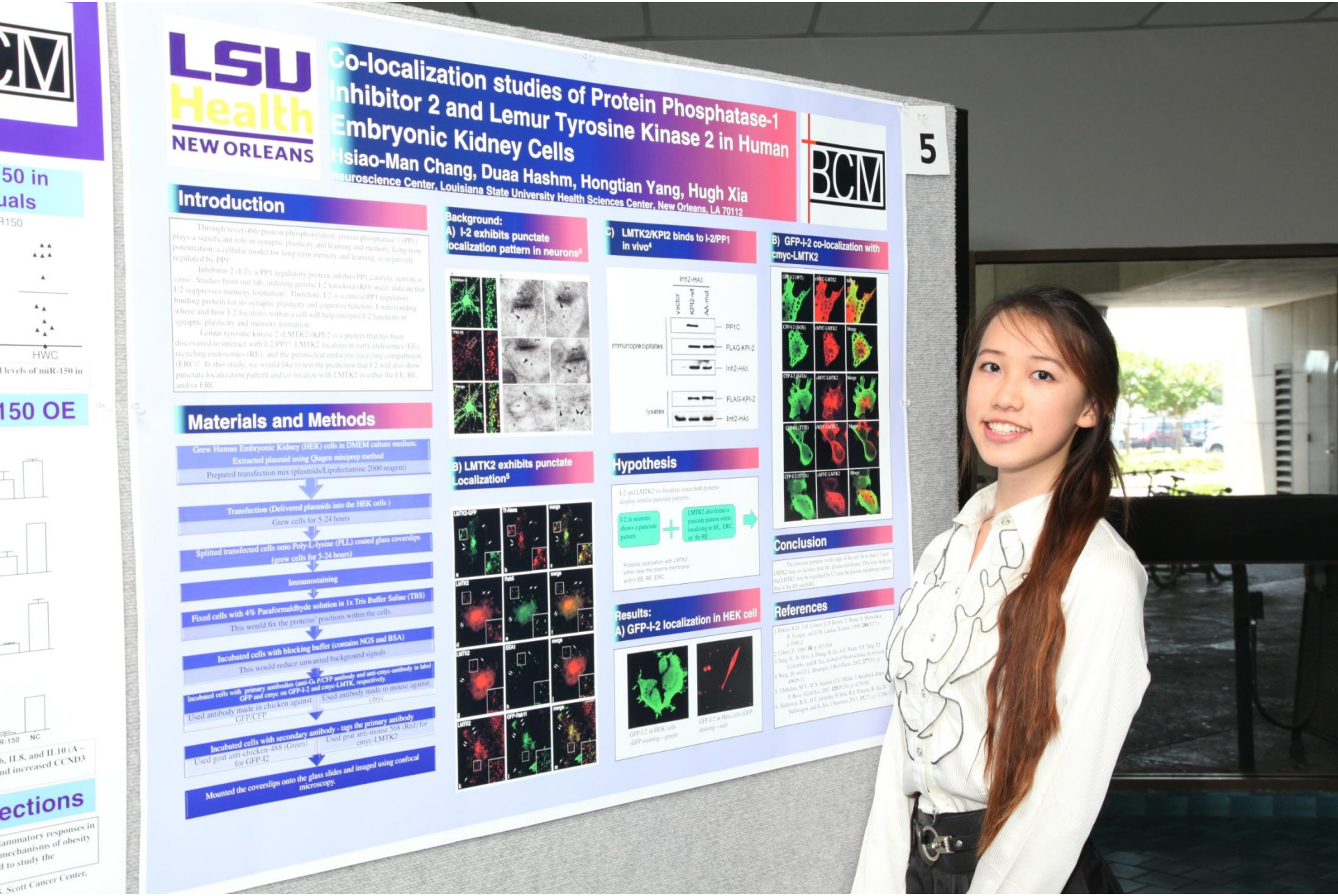
Co-localization studies of Protein
Inhibitor 2 and Lemur Tyrosine Kinase 2 in Human
Embryonic Kidney Cells


LSU Health NEW ORLEANS
Co-localization studies of Protein Phosphatase-1 Inhibitor 2 and Lemur Tyrosine Kinase 2 in Human Embryonic Kidney Cells
Hsiao-Man Chang, Duaa Hashm, Hongtian Yang, Hugh Xia
Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112
BCM
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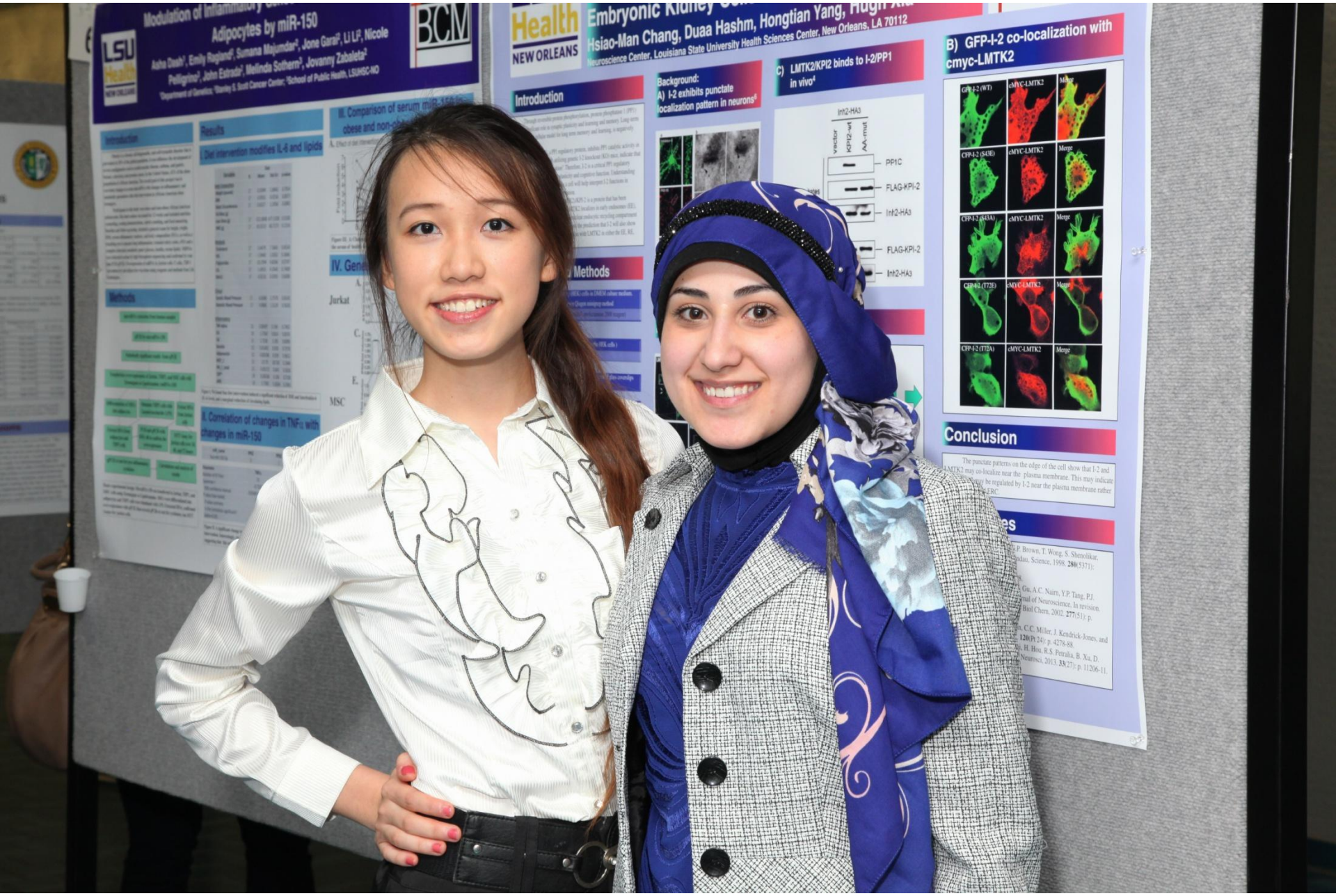
Introduction
Through reversible protein phosphorylation, protein phosphatase 1 (PP1) plays a significant role in synaptic plasticity and learning and memory. Long-term potentiation, a cellular model for long-term memory and learning, is negatively regulated by PP1[1].
Inhibitor-2 (I-2), a PP1 regulatory protein, inhibits PP1 catalytic activity in vitro[2]. Studies from our lab, utilizing genetic I-2 knockout (KO) mice, indicate that I-2 suppresses memory formation[3]. Therefore, I-2 is a critical PP1 regulatory binding protein for its synaptic plasticity and cognitive function. Understanding where and how I-2 localizes within a cell will help interpret I-2 functions in synaptic plasticity and memory formation.
Lemur tyrosine kinase 2 (LMTK2/KPI-2) is a protein that has been discovered to interact with I-2/PP1[4]. LMTK2 localizes in early endosomes (EE), recycling endosomes (RE), and the perinuclear endocytic recycling compartment (ERC)[5]. In this study, we would like to test the prediction that I-2 will also show punctate localization pattern and co-localize with LMTK2 in either the EE, RE, and/or ERC.
Materials and Methods
Grew Human Embryonic Kidney (HEK) cells in DMEM culture medium.
Extracted plasmid using Qiagen miniprep method
Prepared transfection mix (plasmids/Lipofectamine 2000 reagent)
Transfection (Delivered plasmids into the HEK cells)
Grew cells for 5-24 hours
Splitted transfected cells onto Poly-L-lysine (PLL) coated glass coverslips (grew cells for 5-24 hours)
Immunostaining
Fixed cells with 4% Paraformaldhyde solution in 1x Tris Buffer Saline (TBS)
This would fix the proteins' positions within the cells.
Incubated cells with blocking buffer (contains NGS and BSA)
This would reduce unwanted background signals
Incubated cells with primary antibodies (anti-Gα P/CFP antibody and anti-cmyc-antibody to label GFP and cmyc on GFP-I-2 and cmyc-LMTK, respectively.
Used antibody made in chicken against GFP/CFP
Used antibody made in mouse against cmyc
Incubated cells with secondary antibody - tags the primary antibody
Used goat anti-chicken-488 (Green) for GFP-I2
Used goat anti-mouse 568 (Red) for cmyc-LMTK2
Mounted the coverslips onto the glass slides and imaged using confocal microscopy.
Background:
A) I-2 exhibits punctate localization pattern in neurons[6]
B) LMTK2 exhibits punctate Localization[5]
C) LMTK2/KPI2 binds to I-2/PP1 in vivo[4]
Inh2-HA3
vector
KPI2-wt
AA-mut
PP1C
immunoprecipitates
FLAG-KPI-2
Inh2-HA3
lysates
FLAG-KPI-2
Inh2-HA3
Hypothesis
I-2 and LMTK2 co-localizes since both proteins display similar punctate patterns.
I-2 in neurons shows a punctate pattern
LMTK2 also forms a punctate pattern while localizing to EE, ERC, or the RE
Possible localization with LMTK2 either near the plasma membrane and/or EE, RE, ERC.
Results:
A) GFP-I-2 localization in HEK cell
GFP-I-2 in HEK cells (GFP staining—green)
GFP-I-2 in Hela cells (GFP staining—red)
B) GFP-I-2 co-localization with cmyc-LMTK2
Conclusion
The punctate patterns on the right of the cell show that I-2 and that LMTK2 may co-localize near the plasma membrane. This may indicate that LMTK2 may be regulated by I-2 near the plasma membrane rather than in the EE and ERC.
References


Adipocytes by miR-150
BCM
Health
NEW ORLEANS
Hsiao-Man Chang, Duaa Hashm, Hongtian Yang, Hugh
Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112
Introduction
Background:
A) I-2 exhibits punctate localization pattern in neurons
C) LMTK2/KPI2 binds to I-2/PP1 in vivo
B) GFP-I-2 co-localization with cmyc-LMTK2
Results
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