

THE MCNAIR SCHOLARS JOURNAL
OF THE
UNIVERSITY OF CALIFORNIA, DAVIS



Volume XVIII • 2020

RONALD E. MCNAIR



The McNair Scholars Program is a federal TRIO program funded at 187 institutions across the United States and Puerto Rico by the U.S. Department of Education. It is designed to prepare undergraduate students for doctoral studies through involvement in research and other scholarly activities. McNair participants are either first-generation college students with financial need, or members of a group that is traditionally underrepresented in graduate education and have demonstrated strong academic potential. The goal of the McNair Scholars Program is to increase doctoral degree awards for students from underrepresented segments of society.

UC Davis McNair Scholars Program Staff

Program Director and Principal Investigator

Jose Ballesteros, Ph.D.

Program Advisor

Jacques Bowyer, M.S.

Program Specialist

Juan Cervantes

Graduate Student Researchers

Lynette Haberman

Deedee Chao

Journal Editor

Juan Cervantes

Please address inquiries to:

McNair Scholars Program
2300 Student Community Center
University of California, Davis
Davis, CA 95616
(530) 752-7486
mcnair@ucdavis.edu

Message from the Director of the McNair Program

I'm elated to present Volume XVIII of the McNair Scholars Journal of the University of California, Davis. The journal carries on the legacy of Dr. Ronald E. McNair and highlights the achievements of the 2019-2020 cohort and signals their completion of the McNair Scholars Program. As McNair staff, we are given the pleasure of experiencing the scholars in their research training and growth as researchers and professionals. We aim to guide the scholars from finding a research mentor to writing their research manuscript that is represented in the current volume. I want to take a moment to acknowledge the faculty mentors, graduate students, research staff and mentors, Graduate Studies staff, UC Davis staff and Department of Education staff that make the program possible.

Our scholars will go on to graduate school and professional positions and we are excited about what this next generation of scholars will contribute to our society. Congratulations to all of the scholars represented in this journal, we are very proud of you, you carry on the legacy of excellence of Dr. McNair!

*Jose M. Ballesteros, Ph.D.
McNair Scholars Program Director and Principal
Investigator
University of California, Davis*

Table of Contents

Mechanisms that Induce Replication Stress Induced Nucleophagy (ReSIN) Jonathan Do	7
Orphaned Neonatal Kittens Suckling Behaviors and the Implications for Activity Levels and Sleeping Patterns Sabrina Mederos	20
Probing the Hydrogen Bond Network of FUS Fibril Assemblies Rebecca Rafique	37
A case study examining the relation between community health assessments and primary health access for rural communities: Knights Landing, California Sarina Rodriguez	53
Bilingualism and Executive Function: Emerging Bilinguals in Head Start Diana Alicia Santoyo	71
Fibril Formation by the TIA1 Low Complexity Sequence Domain Rachelle Stowell	86
Beyond the Measures Using Mobile Air Sensors to Examine Volatile Organic Compounds in Kettleman City Using a Community-Based Participatory Research Approach Debbie Sulca	101

Table of Contents (continued)

How Genome-Wide Association Studies on Educational Attainment Reify Eugenic Dawn Warfield	117
Locked Down: The Hidden History of the Prisoners' Rights Movement Tiana Williams	130

Mechanisms that Induce Replication Stress Induced Nucleophagy (ReSIN)

Jonathan Do*

Mentor: Dr. Kenneth Kaplan

Department of Molecular and Cellular Biology at UC Davis

Abstract

Cell division, from DNA replication to mitosis, is a highly coordinated process resulting in each cell receiving identical copies of the genome. Genome stability requires coordination between sister chromatid replication and segregation. The final steps in this process occur in anaphase with the resolution of sister chromatids and completion of DNA replication. Failure to properly resolve sister chromatids in a timely manner can give rise to lagging chromosomes that can be inappropriately packaged in nuclear envelope, forming a so-called micronucleus. Micronuclei (MN) are unstable and their disassembly can result in chromosome fragmentation as well as the activation of an inflammatory response triggered by cytosolic DNA. To avoid MN formation, nuclear envelope formation is highly regulated, and it has been proposed that autophagy might surveil and suppress the inadvertent formation of MN. It is unclear how such a surveillance pathway is regulated. We hypothesize that a specific form of autophagy, nucleophagy, is controlled by the Intra-S-Phase kinase cascade that is triggered by changes in chromosome structure that arises from stalled DNA replication. Preliminary findings from our lab demonstrate that the drug hydroxyurea, which depletes nucleotide pools resulting in replication stress, also induces nucleophagy, a pathway we refer to as replication stress induced nucleophagy or, ReSIN. To confirm that ReSIN is triggered by stalled replication forks and not simply depletion

of nucleotide pools, we will engineer cells so DNA polymerase type I can be depleted. We predict that loss of PolI will result in stalled replication forks that will also induce ReSIN and that this pathway will require the intra-S phase signaling pathway.

Introduction

Autophagy is a conserved pathway in which the cell targets organelles or large macromolecular complexes for degradation in the vacuole or lysosome under starvation conditions or after other forms of cell stress. There are three different forms in which autophagy can occur: macro-autophagy, micro-autophagy and chaperone mediated autophagy. In macro-autophagy, organelles (i.e., cargo) are surrounded by a double membrane vesicle called an autophagosome, which is then trafficked to the lysosome or vacuole where the membranes and cargo are subject to degradation. (Kilonsky, 2008). The resulting biochemical building blocks are exported back into the cytosol where they can be re-utilized by metabolic pathways necessary to build new organelles and synthesize proteins. Target of rapamycin, or the TOR kinase, is master regulator of nutrient signaling and regulator of autophagy. During starvation or treatment with rapamycin (a drug that indirectly inhibits Tor kinase activity), inhibition of TOR induces a wide range of autophagy pathways. Mis-regulation of autophagy has been implicated in many clinically relevant diseases from cancer to neurodegenerative disease (Uchiyama et al., 2008).

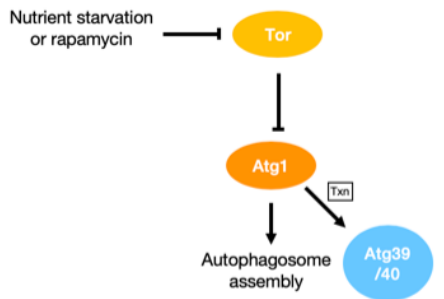


Figure 1: Autophagy Pathway

Figurative diagram of the autophagy pathway. Inhibition of TOR via nutrient starvation or rapamycin allows for activation of Autophagy Gene 1, ATG1, and autophagosome assembly. ATG39/40 are receptors that target the cortical endoplasmic reticulum and the nucleus for autophagy.

Genomic fidelity is critical for the normal function of cells. One critical aspect of this process is that genomes are faithfully replicated and segregated. However, under certain growth conditions or in the presence of cancer chemotherapeutics cells experience replication “stress”, a state that induces a cell cycle checkpoint called the intra-S phase checkpoint. Signals from checkpoint kinases in this pathway serve to stabilize stalled replication forks, arrest cell cycle progression, induce genes regulating nucleotide biosynthesis and ensure that the genome is repaired before moving forward (Iyer and Rhind, 2017). 2017).

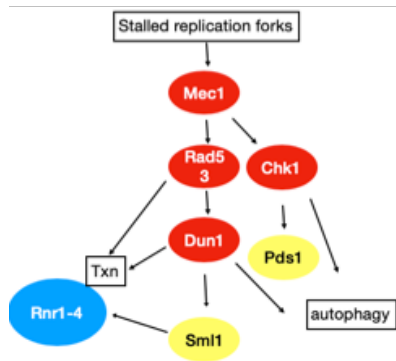


Figure 2: Intra-S-Phase Checkpoint

Figurative diagram of the Intra-S-Phase checkpoint. This pathway is activated when replication fidelity is compromised which is denoted by stalled replication forks. Pds1 halts cell cycle progression once phosphorylated by Chk1. Rnr1-4 encodes for ribonucleotide reductase, an enzyme that produces nucleotides.

Like any other organelle, the nucleus must be maintained in order to ensure proper function. This is especially important for the nucleus because it houses the genome of the organism. The loss of nuclear integrity is associated with multiple diseases such as cancers (Lever and Sheer, 2010). It is well characterized in the literature that lagging chromosomes, or chromosome bridges, in mammalian anaphase cells contribute to MN formation. For example, MN form when topologically linked sister chromatids are prematurely packed and separated into nuclear envelopes. A high frequency of MN is a pathology associated with high chromosome instability and fast evolving tumor cells (Luzha and Kovalchuk, 2013). It has been proposed that formation of MN are suppressed by autophagy pathways that target partially formed nuclei, also known as nucleophagy (Mijalicija and Devenish, 2013). In *Saccharomyces cerevisiae*, the Kaplan lab has observed what appears to be an orthologous nucleophagy pathway that targets nucleolar cargo to the vacuole under replication stress conditions induced by the drug hydroxyurea (Van El Gort et al.).

The nucleophagy pathway has been shown to be under control of the Tor pathway; when Tor is repressed by rapamycin two nucleophagy receptors, Atg39 and Atg40 are up-regulated and also target nucleolar cargo to the vacuole (Mochida et al., 2015) However, in the context of replication stress it is unclear if the same Tor dependent pathway is involved, or whether nucleophagy is induced by the intra-s-phase kinases known to be activated under these conditions. In this study, we set out to test whether stalled replication forks per se, as opposed to Tor dependent nutrient signaling, activates ReSIN and whether the activation signals depend on the intra S-phase checkpoint pathway.

Material and Methods

Cultivation of Yeast

Yeast were cultivated on yeast peptone dextrose (YPD) agar plates for two days in a 30°C growth chamber. If the yeast contained an autonomously replicating plasmid with a selectable marker, then they were plated on a drop out (SD) plate to ensure plasmid integrity. For yeast cell lines that were integrations, they were plated as normal. To prepare for genomic extractions, microscopy experiments, or western blots, a sterilized wooden stick was used to pick up a colony of yeast and inoculate in 5mL of liquid media. However, for microscopy experiments, YPD was not used. A dropout media (SD) replenished with complete amino acids were used instead to avoid auto-fluorescence. Target concentrations of cells/mL of liquid culture varied on the type of experiment.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was used in order to amplify the desired target locus. Forward and reverse primers were designed on serial cloner based on the genomic sequence from SGD. For a two-reaction genomic PCR, 134uL of sterile H₂O, 40uL 5X HF buffer, 16uL 2.5mM dNTPs, 2uL each of forward and reverse primers, 4uL of genomic DNA and 2uL of Phusion enzyme. For a two-reaction plasmid PCR, 118uL of sterile H₂O, 40uL 5X HF buffer, 16uL 2.5mM dNTPs, 2uL each of forward and reverse primers, 20uL of plasmid diluted 1:1000 in Tris buffer, and 2uL of Phusion enzyme. Everything was prepared on ice to prevent non-specific amplification. The master mix was then pipetted into 100uL aliquots into reactions tubes before placed into the heated machine.

Table 1: PCR protocol for *GAL1p-POLI*. Annealing temperature will vary based on the primers and extension time will be dependent on size of the targeted locus.

Cycles	1		25		1	
Temp (°C)	Denaturing 98	Denaturing 98	Annealing 57.3	Extension 72	Extension 72	Cooldown 4
Time (mins)	5:00	0:30	0:30	1:45	10:00	hold

Confirmation of PCR Product

In order to confirm desired amplification of the target locus, a 1% agar, 1:20000 gel green and 200mL solution of 1X TBE was melted and poured into a lane cast. The gel was allowed to solidify before being subjected to gel electrophoresis of 50V for one hour. The buffer for the electrophoresis treatment was 1X TBE

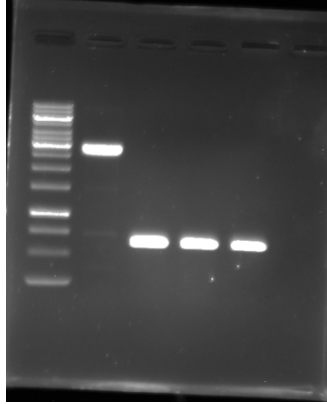


Figure 3: PCR screening of ATG39-GFP Transformation

The lane furthest to the left is the ladder, following that is the positive control and the subsequent cell lines being tested for presence of locus integration which was not successful.

Yeast Transformation

Yeast were inoculated in 5mL of YPD liquid media at 30°C, the day before allowing growth. They were then diluted to 0.5×10^7 cells/mL and allowed to grow for three hours at 30°C, 2 doublings, to achieve the final concentration of 2.0×10^7 cells/mL and to assure that the yeast were in logarithmic phase. They were then transferred into a disposable 15mL conical and centrifuged at 3k RPM for 10 mins. The supernatant was disposed and the pellet resuspended in 5mL of sterile water before being centrifuged once more. The water was then removed, and the pellet resuspended in 100mM lithium acetate (LiAc) before being transferred to a 1.5mL Eppendorf tube. The cells were spun and 14kRPM for 15 seconds and resuspended in 4x cell volume of 100mM LiAc. The cells were then vortexed and pelleted before adding 50uL of LiAc and transferred into transformation tubes. The LiAc was removed and yeast were subjected to 360uL the transformation mix (TRAFO Mix) which consisted of: 240uL 50% Polyethylene glycol, 1M LiAc, 20uL PCR/Plasmid Product with a

concentration of .25ug/uL, 50uL salmon sperm DNA and 14uL of sterile H₂O. The pellet was vortexed thoroughly to ensure proper mixing. The mixture was then subjected to a 30°C water bath for 30mins then transferred to a 42°C water for 30mins. The mixture was spun at 5k RPM for 15s and the supernatant removed. The pellet was resuspended in 200uL of sterile water and gently mixed to prevent cell lysis. The mixture was plated onto appropriate drop out plates and incubated for two days at 30°C.

Rapamycin

Yeast were grown to a concentration of 1.00×10^7 cells/mL in 5mL of liquid media and the bud index measured to assure that the culture was in log phase which was denoted by a bud index of around 50%. The culture was then treated with rapamycin to achieve a final concentration of 200uM. The culture was then incubated at 30°C for three hours before analysis.

Hydroxyurea

Yeast were inoculated overnight in 5mL of appropriate liquid media at 30°C. They were then counted, diluted back to 0.25×10^7 cells/mL, and counted after three hours to achieve the target concentration of 1.0×10^7 cells/mL. They were counted again, and the bud index measured to assure at least 50% of cells were budded to indicate log phase. The cultures were split into two and one was treated with 1M hydroxyurea such that the final concentration of hydroxyurea was 0.1M. They were then allowed to grow for three hours at 30°C and counted to assure >80% budded to indicate successful replication stress. The cells were pelleted, washed three times with sterile water and resuspended in media. Nocodazole (15mg/mL) was added to both the treated and untreated to achieve the final concentration

of 15ug/uL. The cells were allowed to grow for one hour before being washed three times with sterile water. Four percent low melting agarose pads were prepared and 2uL of culture was added and sealed with vLAP before imaging via microscopy.

Galactose Inducible Polymerase

Yeast with the *Galp-POL1* construct were cultured in 5mL SD 2% Raffinose/ Galactose media. These were grown up overnight, counted to make sure they were in log phase, diluted to a concentration of 0.25×10^7 cells/mL and switched into 5mL SD 2% Dextrose media. The media was changed every two hours to prevent starvation.

Statistical Significance

In order to determine whether the numbers collected were significant, a two-tailed student T-test was applied in excel using the mean of taken fields. The means of each strain at each timepoint were then tested against one another. A value of $P < 0.005$ was determined to be statistically significant.

Results

GAL1p-POL1 induces cell cycle arrest consistent with stalled DNA replication

It is well established that depletion of nucleotides via the drug hydroxyurea arrests cells in early S phase, an arrest that causes budding yeast to accumulate in a large budded state. To test the efficacy of *Galp-POL1* in engaging the intra-S phase checkpoint, we monitored the bud index of cells grown under repressive conditions (i.e., dextrose in the media) to deplete the Pol1 polymerase. Because the Pol1 protein is quite stable it requires “dilution” during cell division to be depleted and thus cause cell cycle arrest (Falconi et al., 1993). Consistently, we

observed an increase of large-budded cells after 5 to 7 generations or 10-14hrs after switching cells into dextrose containing medium. This is consistent with the induction of replication stress and stalled replication forks after depletion of Pol1.

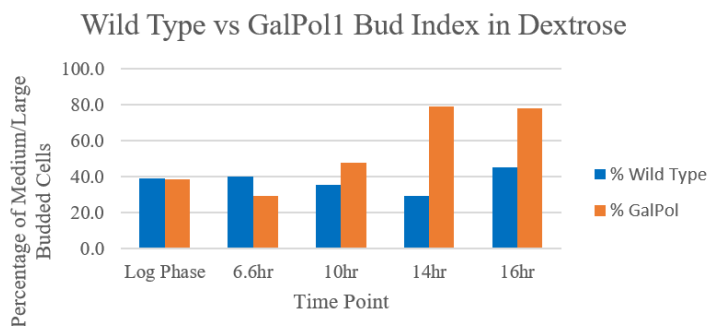


Figure 4: Wild Type and GalPol1 cultures cultured in dextrose
 During Log phase, cultures were grown in SD uracil dropout with 2% raffinose and galactose. For the following timepoints, the cultures were grown in SD uracil 2% dextrose

GAL1p-POL1 are normal for rapamycin induced macroautophagy

To monitor nucleophagy induced by replication stress, GFP-Atg8 was used to mark the formation of the so-called phagore-assembly site, or PAS, where autophagosomes are assembled. We first showed that the *GAL1p-POL1* cells are able to induce PAS normally by treating them with rapamycin to inhibit Tor and induce bulk autophagy. When cultures were treated with rapamycin, we indeed saw an increase in foci compared to normal, vegetative growing cells, indicating that these cells have fully functional autophagy pathways.

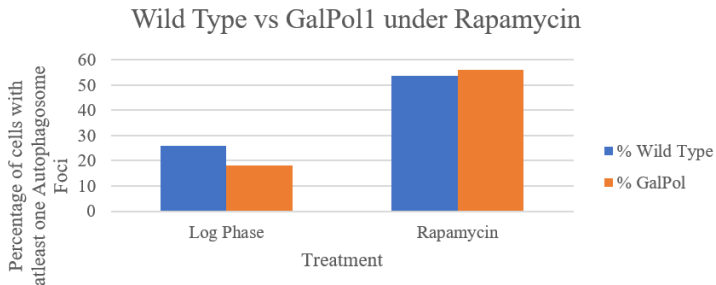


Figure 5: Wild Type and GalPol1 treated with Rapamycin

During Log phase, cultures were grown in SD uracil dropout with 2% raffinose and galactose. For Rapamycin treatment, the cultures were grown in log phase in SD uracil dropout with 2% raffinose and galactose then treated to achieve a final concentration of 200uM and incubated for three hours.

GAL1p-POL1 inhibition increase GFP Atg8 marked PAS

Using the conditions that we established to cause activation of the intra-S phase checkpoint and cell cycle arrest, we monitored the kinetics of PAS formation. We cultured cells where *POL1* is expressed under its native promoter simultaneously with the *GAL1p-POL1* repressible cell line under repressive conditions. We expected to see a PAS formation starting 14hrs of transcriptional repression based on our cell cycle experiments. Consistent with this expectation, we observed a statistically significant ($p=0.04813$) increase in PAS that peaked after 16hours. We speculate that the increase in PAS observed at the six-hour time point is due to poor culture conditions for that single *Galp1-POL1* sample. In conclusion, this data supports our hypothesis that ReSIN is induced by stalled replication forks and not by depletion of nucleotide pools caused by the cancer chemotherapeutic, hydroxyurea.

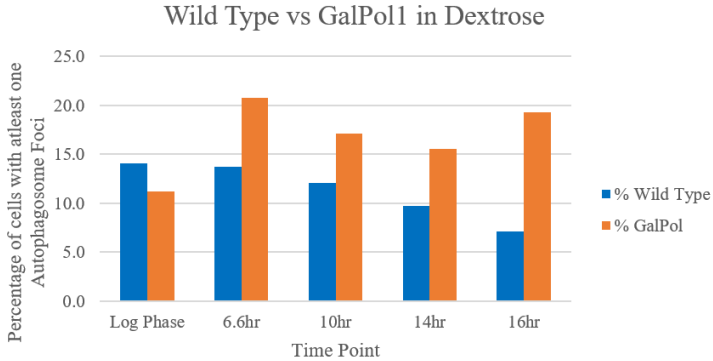


Figure 6: Wild Type and GalPol1 cultures cultured in dextrose

During Log phase, cultures were grown in SD uracil dropout with 2% raffinose and galactose. For the following timepoints, the cultures were growing in SD uracil 2% dextrose. However, at 6.6hrs, the GalPol1 culture was left in hypoxic conditions possibly explaining the increase.

Discussion

In this study, we showed that replication stress can be induced by inhibiting transcription of the *POL1* gene and the resulting depletion of Pol1 protein from cells. Significantly we found that cell cycle arrest due to Pol1 depletion was accompanied by an increase in ReSIN as measured by the increase in PAS and autophagosome formation. Though the kinetics of PAS formation due to ReSIN are delayed compared to what we observed with the drug hydroxyurea, this is expected given the slow kinetics of polymerase depletion compared to nucleotide depletion. These initial results are consistent with the prediction that replication stress, whether induced by a reduction in polymerase or nucleotide pools, can activate an autophagy pathway. This finding further supports the hypothesis that intra S-phase signaling is involved in replication stress induced nucleophagy. In combination with previous observations from our lab that the intra-S-phase checkpoint kinases are necessary for autophagic flux after

nucleotide depletion, we conclude that there is a novel branch of this checkpoint that induces a replication stress induced form of nucleophagy, or ReSIN.

This conclusion raises a number of very interesting questions. How do the intra S-phase kinases mediate the induction of nucleophagy? One possibility is that these checkpoints induce a transcriptional response that helps to activate nucleophagy specifically. This idea is consistent with observations in rapamycin treated cells where the ER-nucleophagy receptors Atg39 and Atg40 levels are increased (Mochida et al, 2015). What is the relevant cargo that is being targeted by ReSIN and how does it contribute to genome instability? Our observations that nucleolar proteins appear to be cargo during ReSIN raises the possibility that stalled replication places a stress on the nucleolus, a sub-compartment of the nucleus that houses the rDNA array. The rDNA array is highly repetitive and thus difficult to replicate and is a hot spot of transcription, creating an additional challenge for the replication machinery to copy this region in a timely manner. Finally, the fact that rDNA containing chromosomes are some of the last to resolve in anaphase raises the intriguing possibility that ReSIN is tied to its timely resolution and the suppression of these unresolved loci contributing to genome instability.

Acknowledgments

I would like to thank the McNair Scholars program for giving me opportunities and confidence to conduct independent research. I would especially like to thank Dr. Kenneth Kaplan for his advice, guidance, and support during these experiments.

References

- Falconi, M. M., Piseri, A., Ferrari, M., Lucchini, G., Plevani, P., & Foiani, M. (1993). De novo synthesis of budding yeast DNA polymerase α and POL1 transcription at the G1/S boundary are not required for entrance into S phase. *Proceedings of the National Academy of Sciences of the United States of America*.
- Iyer, D. R., & Rhind, N. (2017). The intra-S checkpoint responses to DNA damage. *Genes*.
- Klionsky, D. J. (2008). Autophagy revisited: A conversation with Christian de Duve. *Autophagy*.
- Lever, E., & Sheer, D. (2010). The role of nuclear organization in cancer. *Journal of Pathology*.
- Luzhna, L., Kathiria, P., & Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: From genetics to epigenetics and beyond. *Frontiers in Genetics*.
- Mijaljica, D., & Devenish, R. J. (2013). Nucleophagy at a glance. *Journal of Cell Science*.
- Mochida, K., Oikawa, Y., Kimura, Y., Kirisako, H., Hirano, H., Ohsumi, Y., & Nakatogawa, H. (2015). Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature*.
- Uchiyama, Y., Shibata, M., Koike, M., Yoshimura, K., & Sasaki, M. (2008). Autophagy-physiology and pathophysiology. *Histochemistry and Cell Biology*.
- Van Elgort, A., Perucho-Jaimes, L., Do, J., & Kaplan, K. (Forthcoming). Nucleophagy

Orphaned Neonatal Kittens Suckling Behaviors and the Implications for Activity Levels and Sleeping Patterns

Sabrina Mederos*

Mentor: Mikel Delgado, PhD; Tony Buffington, PhD, DVM
Department of Medicine and Epidemiology at UC Davis
School of Veterinary Medicine

Abstract

*Behavioral differences between neonatal kittens (*Felis catus*) raised in foster homes and those receiving direct maternal care may lead to issues that jeopardize welfare and development. Maternal interactions involving nursing and weaning have far-reaching effects on behavioral and physical development of neonatal kittens. One behavior routinely observed in foster homes and shelters has been found to pose a concern to the health of kittens: non-nutritive suckling (NNS). In this observational study, video footage of 15 litters of orphaned neonatal kittens raised and bottle fed by human foster caretakers was assessed to identify if there was a relationship between the presence of NNS (oral contact to littermates' bodies that is not for the purpose of nutrient consumption) and quantified time engaged in activity throughout a typical day. This was done to assess if there is a trade-off between the motivation to engage in NNS and sleep during typical rest periods. Additionally, supplemental data from an online survey provided complementary information regarding temporal and spatial patterns associated with suckling in kittens who engage in NNS. By exploring the relationship between neonatal suckling due to a lack of a maternal presence and potential effects on activity level (and presumably, sleep), we can*

determine additional ramifications that being orphaned may have on the development of abnormal behaviors, and perhaps how to better address the welfare issue NNS poses for orphaned kittens.

Introduction/Background

Feline maternal behavior and care of offspring have been extensively studied, but comparatively, fostered/hand-reared orphaned neonatal kittens are less well understood. Because of our general understanding of kitten health, the capability to address many of the physiological concerns normally managed by a mother cat has greatly improved, although successful management must consider both physical and behavioral factors involved in the care of neonates (Little, 2013). There is relatively limited research that assesses differences in behavior between neonatal kittens hand-reared by humans and those raised by a queen.

As a mammalian species, lack of maternal care prompts a need for comparative evaluation of the possible variability exhibited when kittens are orphaned. The physical needs of orphaned kittens (such as immunoprotection, adequately warm housing, and around the clock care) can be attended to given the current understanding of kitten physiology, but fulfilling the behavioral niches that a mother provides does not always prove viable (Snook & Riedesel, 1987). Evidence of the importance of this behavioral niche can be seen in the drastic developmental implications associated with the timing and nature of the weaning process a litter is subject to when kittens experience withdrawal from maternal care (Martin, 1986). The effects of premature withdrawal of maternal interaction during weaning can be physiological or behavioral but largely they are detrimental (Seitz, 1959).

Many behavioral abnormalities have been observed in human-reared kittens, including inappropriate responses to social interactions, abnormal sexual behavior, exaggerated aggressive tendencies, and evidential signs of compromised health (Hart, 1972; Ahola, et al., 2017); these abnormalities are often attributed to the lack of maternal care. Scientific consensus is that early-life maternal-offspring interactions affect the behavioral, physical and emotional development of offspring well into adulthood (Beaver, 2003; Hart, 1972). Studies in domestic cats have directly tested this, including one that recorded aberrant behaviors exhibited in high frequencies in kittens isolated from their mothers and littermates (Konrad & Bagshaw, 1970).

Behavior can indicate underlying emotional anomalies; both possibly having biologically relevant health consequences. This is evident in the population of kittens from which this project was derived (investigating the role temperature and relative humidity may play in the health of orphaned neonatal kittens). Of the 68 foster kittens, 23 were found to be impacted by NNS; 9 of 23 litters were impacted (40%); 17 kittens were “suckers”; 17 kittens were “victims”; and 11 were classified as both. Kittens engaged in up to 25-80 bouts of sucking per day, sucking for up to 56-135 minutes each day. This prompts an exploration of other risks factors involved and what may be the driving force behind the occurrence of NNS.

NNS is not exclusive to neonatal kittens in foster settings; it has been investigated in a variety of mammalian species such as pigs (Rushen & Fraser, 1989; Bøe & Jensen, 1995); horses (Tyler, 1972; Crowell-Davis, 1985); cows (Lidfors et al. 1994; Rushen & de Pasillé, 1995); and even human infants (de Carvalho et al., 1982). NNS has previously been identified in mother-reared cats (Koepke & Pribram 1971), though the behavior in that case was directed to a provided artificial

nipple. For many foster caretakers, the behavior is classified as an issue to monitor for as it poses a possible risk to the litter being cared for. Many of the proposed solutions for mitigating the occurrence of NNS in other species in commercial settings involve providing alternative outlets for non-nutritive sucking and similar motor patterns, such as environmental enrichment or novel feeding methods (Widowski, et al., 2005; Horvath & Miller-Cushon, 2017; Bench & Gonyou, 2006). These interventions aim to redirect the behavior, but do not address the underlying motivation behind it, largely because nursing behaviors are not driven strictly by physical needs (such as consumption of nutrients) but also emotional and social needs (Carson & Wood-gush, 1983).

Previous studies of mammals have identified suckling and other motor patterns associated with nursing behaviors as serving many purposes other than consumption of nutrients. Suckling itself is a form of social interaction between conspecifics (Robbin & Moen, 1975; McVittie, 1978; Li & Gonyou, 2002) and NNS may compensate for a lack of maternal social interaction. Two studies looked at the differences between litters of kittens raised by a mother versus a brooder (a nonsocial stimulus); littermates raised on the brooder would often nuzzle or suck one another to the point of hair loss (Schneirla, et al., 1963; Guyot, et al., 1980).

In some contexts, suckling may be a form of stereotypic behavior. Stereotypies are common in a vast number of species and are broadly defined as a repetitive, seemingly functionless, sequence of actions not commonly exhibited in wild populations (Mason, 1991a). These patterns are most prominently evident in captive settings where animals have little to no control over their environment, thus indicating a need for a sort of self-soothing mechanism (Luescher, et al., 1991). Suckling on litter mates may be adaptive by eliciting

physiological changes such as decreased heart rate, or changes to digestion and activity levels. A study done on rats found suckling elicits an analgesic response (Anseloni, et al., 2004). Suckling has reduced heart rate during stressful procedures in various mammals, such as calves (Veissier, et al., 2002) and even humans (Dipietro, et al, 1994). Additionally, animals suckle when distressed or alarmed, providing further evidence that suckling may prove an outlet for stress as well as a method of nutrient consumption (Lent, 1971).

NNS may satisfy a reflexive or evolutionarily developed need considering that mammals are highly motivated to perform oral behaviors that are innate to their species (de Passillé, 2001; Widowski, et al., 2008). The feeding behaviors of neonates are largely driven by maternal initiation and innate reflexes (Snook & Riedesel, 1987). The “rooting reflex” is driven by young kittens’ inability to regulate homeostatic functions, so they huddle toward their mother or littermates for warmth; huddling continues for up to 16 days of age (Beaver, 2003). Sucking is a reflex present at birth and is initiated through a number of proposed stimuli, such as the oral-tactile stimulation of small objects or a hairless area (Ewer, 1959; Beaver 2003). Furthermore, another study established that while development of sucking from a mother’s nipple quickly follows birth, attachment and consumption from an artificial nipple requires learning, and failure to successfully feed from an artificial source can be a mortality risk (Kovach & Kling, 1967).

If orphaned kittens are spending substantial time either initiating or receiving suckling from littermates, there may be an inherent trade-off with other behaviors. Sleep is essential to the development and well-being of neonatal animals and a lack of sleep can be very detrimental to health. Increased external stimulation may decrease restful sleep for kittens; periodical incidences of suckling of littermates could disrupt quality and

quantity of sleep (Serman, et al., 1965; Beaver, 2003). When forced to stay awake for extended periods of time, cats will become increasingly irritable, even to the point of illness, but effects of sleeplessness on learning are not conclusively defined (Vogel, 1975; Beaver, 2013). The long-term effects of inadequate sleep on the growing kittens are unknown. In human infants and kittens sleeping patterns are developed in younger ages (Chase & Serman, 1967). A major developmental period for diurnal sleep/waking rhythms in cats is between 4-6 weeks of age and begin to take on adult configurations at about 90 days (Hoppenbrouwers & Serman, 1975). Maturation of particular brain areas (basal forebrain) that regulate sleep cycles is required for development of these rhythms, so until then their sleep pattern is plastic; compromising effects to sleep during this period could potentially be harmful later on in life (Stern, et al., 1973).

The aim of this study is to investigate the effects of NNS behavior on activity levels. We quantified the percentage of time (within total time observed) for each day that each litter engaged in activity and compared that between litters that had suckers present and litters where sucking was not observed. We hypothesized that sucking litters will have higher percentages of their daily time budget dedicated to activity because they have exhibited NNS during periods that those who don't suck might spend sleeping or resting. From our observations, NNS includes search and escape behaviors that we believe increase overall activity. Furthermore, upon assessing if activity increases as the kittens age, we hypothesized that kittens would be more active as they matured.

Methods

All animal procedures were approved by the Animal Care and Use Committee at the University of California, Davis. A survey

of foster caretakers was approved by the Institutional Review Board of the University of California, Davis. The kittens in this study were part of a larger project that required they be housed in commercial incubators (Rcom MX BS600N) and continually filmed. The kittens were cared for in foster homes after being surrendered to local rescue organizations. Because of this, each of the 15 litters was cared for in adherence to guidelines and expectations set by the experimenters and the rescue organizations.

The 15 litters ranged in size from 2 to 5 kittens, and ages from 1 to 25 days at the start of the video footage. Kittens were recorded for varying amounts of time, and the footage continued until the kittens were ages 11 to 32 days. There were 33 males, and 21 females totaling to 54 kittens.

The access to 24-hour video footage allowed us to obtain an accurate representation of kittens' behavior throughout the day. Because coding of video is time-consuming, we automated the collection of data. A command-line application in Python (DVR-Scan) was used to scan all video files collected; comparing frame-by-frame it detects pixel changes above a defined threshold. A code script in R provided output of the start and end time of continuous pixel changes detected and calculated the total amount of movement (in seconds) displayed by the kittens within each video. To compare activity and inactive time, we considered activity to be any movement displayed by any kitten in the video as quantified through the software.

To ensure reliability of this automated process, we took 12 sample hours of video, ran them through the program and compared the results to hand-coded results for the same videos. Both measures provided the total number of seconds the kittens were active in a given video. From this we established a

Pearson's correlation coefficient of $r = 0.93$, proving strong evidence for the reliability of this method. To account for the fact that these kittens may be fed throughout the day and to ensure the movement detected was only movement performed by the kittens, we omitted feeding times manually, which was done by referring to records provided by foster caretakers and rendering the time in which there was feeding related movement on the footage exempt. After omitting the feeding times from the overall daily time budget, we calculated the percentage of time each litter spent engaged in activity of the total time observed.

Knowing that the amount of activity displayed within each litter was dependent on a number of different variables, for our preliminary runs we compared data from litters of similar compositions, varying only in respect to the presence of sucking behaviors; number of kittens within the litter and age at the time of footage used would be held consistent. We would then compare the percentages to see which litter exhibited heightened levels of activity and if these amounts changed with age. For further analysis we coded all available footage and conducted analyses in R 3.6.0 (Foundation for Statistical Computing, Vienna, Austria). We analyzed the effect of age, litter size (LS) and sucking (Y/N) using a linear mixed model with litter identity as a random effect to control for repeated measures.

To additionally understand NNS, a survey comprising of 60 questions was distributed online to households fostering orphaned litters exhibiting suckling behavior. We collected data from 331 litters which included 1,106 kittens. From this data, we obtained information about temporal and spatial patterns of suckling, identification of the presence of suckling behaviors, and strategies for the mitigation of NNS.

Results

The first preliminary analysis was a comparison between litter A, which composed of two kittens, 11 days of age during the 48 hours pulled and had sucking present, and litter B, same number of kittens and same age, but displaying no NNS. Litter A spent 55% of the time accounted active whereas litter B spent 38% of their time active. For the second comparison, we looked at two litters that had four kittens that were 13 days of age; litter C had sucking present and they spent 55% of their time active. Litter D did not exhibit sucking and spent 63% of their time active.

After analysis of all the video footage we found no correlation between NNS and activity. There was no significant relationship between sucking behavior and activity ($F(1, 11.2) = 1.40, p = 0.26$), Figure 1). There was also no relationship between litter size and the time spent active excluding the presence of sucking ($F(1, 10.5) = 1.00, p = .34$, Figure 2). However, analysis of the relationship between age of the kittens and activity showed that as the kittens matured, activity level appeared to decrease ($F(1, 128)=44.10, p < .001$, Figure 3).

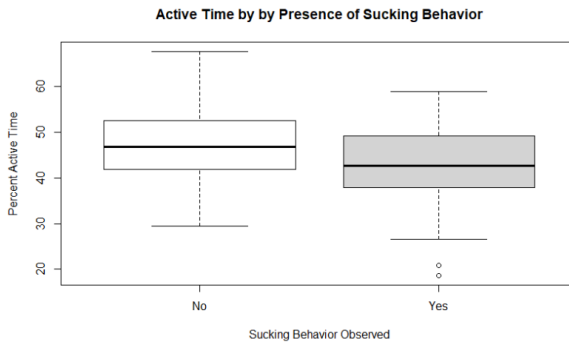


Figure 1: Active Time by Presence of Suckling Behavior

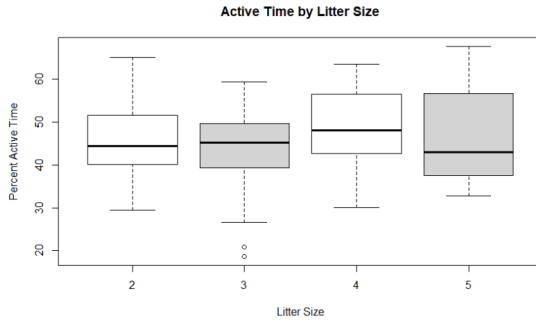


Figure 2: Active Time by Litter Size

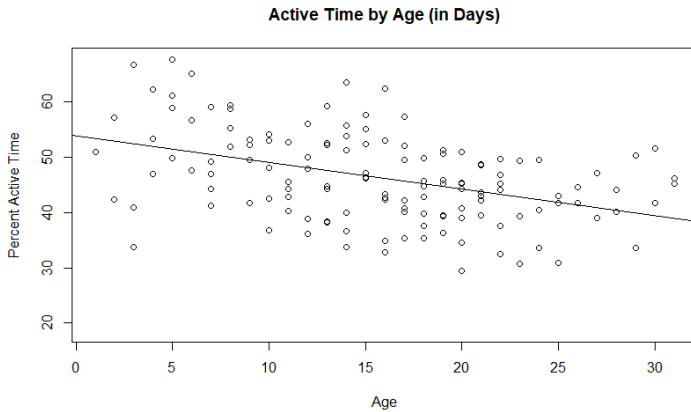


Figure 3: Active Time by Age

The survey data revealed the most common ways that sucking was identified was direct observation as it was happening (81% of respondents) and observation of wet areas on the victim's bodies (58%). Less common ways were seeing wet areas on the sucker's face (26%), or from the presence of sores (16%).

Survey data showed 63% of victims were male, and a chi-squared test revealed that males were more likely to be a victim of sucking ($X^2(2) = 22.16, p < .001$). No significant effect was found of sex on initiating sucking. From data of litters where weight information was included, the largest kitten was a victim of sucking in 59.7% of litters, and in 58.4% of the litters, the smallest kitten was a victim.

To mitigate sucking behavior, many foster caretakers stated they interrupted the behavior (68%) and often separated the kittens part time (42%), but others reported using clothing or even separating the kittens full-time (20%). Participants also noted that a majority of the kittens that were separated, reinitiated sucking when brought back together. The majority of participants (76%) indicated they fed the kittens according to a set schedule. Information on when the sucking was occurring relative to sleep/wake periods suggested that suckers often slept following a bout of sucking whereas for victims sleep periods were much more variable.

Discussion

Both the preliminary results and the analysis on the entire set of video footage gathered tells us that there was no conclusive correlation between activity and the presence of sucking. Therefore, we cannot assume sucking is associated with heightened activity or reduction in the quantify of restful, non-movement periods. Regardless, in a number of instances we observed a disruption of sleep prompted by the initiation of sucking from one kitten onto another, but it is possible this trade-off is compensated for in other periods of time. Kittens could potentially be exhibiting longer periods of sleep in less frequent bouts as a result of exhaustion from sucking, therefore overall be active the same amount as non-suckers. To analyze this, we would need to examine each bout of sucking and

compare it to bouts of sleeping to see if there is any relationship there.

Furthermore, we expected litter size to have a significant effect on activity, such that the more kittens there were in a litter, the more potential for interactions to be had. We did not find that to be the case. This is likely due to the method we use for data collection posing as a potential limitation to the study. The software measures activity through pixel changes between frames, meaning any movement detected counts as movement for all kittens present, not on an individual basis. Therefore, each litter is treated as a single unit and the number of kittens is unaccounted for outside of data analysis.

Because activity is quantified as movement detected through pixel changes, it is possible that pixel changes could be attributed to other things in the camera's view. We attempted to account for that limitation by using the reported feeding times to omit times where the camera would have caught other movements. Lastly, we expected that as the kittens aged, they would spend more time active. Although the video footage primarily focused on kittens who are not fully mobile, as their senses become more adept at sensing their surroundings and as motor activity and coordination increases, we would expect more movement. Our data showed the opposite to be true; across litters, as time progressed the kittens were significantly less active. This could potentially be a result of adjustment to surroundings, as kittens became more familiar with their environment, they may have been less motivated to engage in exploratory behaviors and more likely to rest. Increasing sample size and looking at a more diverse group of kittens may shed light on this finding.

Conclusion

The results prompt many more questions about the implications of NNS on the development and behavior of kittens. If the trade-off in time isn't with sleep, then what behaviors are occupying the time non-suckers would be using to suck? If the difference is accounted for in bout frequency what are the long-term effects of sleep deprivation on developing young from inconsistent sleep? Are there possible differences and notable outcomes expressed in adulthood as a result of NNS?

Age, litter size, individual variation, feeding amount and frequency all have effects on the amount of activity kittens might exhibit. We hope to do in depth analysis of specific trends in suckling/sleeping patterns such as sex differences, external risk factors, bout frequency, and victim vs. initiator analysis. Within the future many more orphaned kittens will be in need of homes allowing us the opportunity to increase our sample size or conduct further studies to evaluate this abnormal behavior.

References

- Ahola, M. K., Vapalahti, K., & Lohi, H. (2017). Early weaning increases aggression and stereotypic behaviour in cats. *Scientific Reports*, 7, 1-9. doi:10.1038/s41598-017-11173-5
- Anseloni, V., Ren, K., Dubner, R. & Ennis, M. (2004). Ontogeny of analgesia elicited by non-nutritive suckling in acute and persistent neonatal rat pain models. *Pain*, 109, 507–513.
- Beaver, B. V. (2003). *Feline Behavior* (2nd ed.). Elsevier Saunders.
- Bench, C.J. & Gonyou, H.W. (2006). Effect of environmental enrichment at two stages of development on belly nosing in piglets weaned at fourteen days. *Journal of Animal Science*, 84, 3397–3403.

Bøe, K., & Jensen, P. (1995). Individual differences in suckling and solid food intake by piglets. *Applied Animal Behaviour Science*, 42, 183-192.

Carson, K. & Wood-Gush, D. G. M. (1983). Equine behaviour: I. A Review of the literature on social and dam-foal behaviour. *Applied Animal Ethology*, 10, 165-178.

de Carvalho, M., Robertson, S., Friedman, A., & Klaus, M. (1982). Milk intake and frequency of feeding in breast fed infants. *Early Human Development*, 7, 155-163.

Chase, M. H., & Serman, M. B. (1967). Maturation of patterns of sleep and wakefulness in the kitten. *Brain Research*, 5, 319-329.
doi:10.1016/0006-8993(67)90040-6

Crowell-Davis, S. L. (1985). Nursing behaviour and maternal aggression among Welsh ponies (*Equus caballus*). *Applied Animal Behaviour Science*, 14, 11-25.

DiPietro, J.A., Cusson, R.M., O'Brien Caughy, M. & Fox, N.A. (1994). Behavioural and physiologic effects of nonnutritive sucking during gavage feeding in preterm infants. *Pediatric Research*, 36, 207-214.

Ewer, R. F. (1959). Suckling Behaviour in Kittens. *Behaviour*, 15(1-2), 146-162. doi:10.1163/156853960x00142

Guyot, G. W., Cross, H. A., & Bennett, T. L. (1980). Early social isolation of the domestic cat: Responses to separation from social and nonsocial rearing stimuli. *Developmental Psychobiology*, 13(3), 309-315. doi:10.1002/dev.420130306

Hart, B. L. (1972). Maternal Behavior II - The nursing sucking relationship and the effects of maternal deprivation. *Feline Practice*, 2, 6-8.

- Hoppenbrouwers, T., & Sterman, M. B. (1975). Development of sleep state patterns in the kitten. *Experimental Neurology*, 49(3), 822-838.
- Horvath, K. C., & Miller-Cushon, E. K. (2017). The effect of milk-feeding method and hay provision on the development of feeding behavior and non-nutritive oral behavior of dairy calves. *Journal of Dairy Science*, 100(5), 3949-3957. doi:10.3168/jds.2016-12223
- Hudson, R., & Distel, H. (2013). Fighting by kittens and piglets during suckling: What does it mean? *Ethology*, 119(5), 353-359.
- Koepke, J. E. & Pribram, K. H. (1971). Effect of milk on the maintenance of sucking behavior in kittens from birth to six months. *Journal of Comparative and Physiological Psychology*, 75, 363–373.
- Konrad, K. W. & Bagshaw, M. (1970). Effect of novel stimuli on cats reared in a restricted environment. *Journal of Comparative and Physiological Psychology*, 70(1), 157-164.
- Kovach, J.K. & Kling, A. (1967). Mechanisms of neonate sucking behavior in the kitten, *Animal Behaviour*, 15(1), 91–101.
- Lent, P. C. (1971). Mother-infant relationships in ungulates. In *The Behaviour of Ungulates and its Relation to Management* (Vol. 1, pp. 14-45). Morges: IUCN Publications.
- Lidfors, L. M., Jensen, P. & Algers, B. (1994). Suckling in free-ranging beef cattle: temporal patterning of suckling bouts and effects of age and sex. *Ethology*, 98, 321–332.
- Little, S. (2013). Playing Mum: Successful management of orphaned kittens. *Journal of Feline Medicine and Surgery*, 15(3), 201-210
- Li, Y.Z. & Gonyou, H.W. (2002). Analysis of belly nosing and associated behaviour among pigs weaned at 12–14 days-of-age. *Applied Animal Behavior Science*, 77, 285–294.

Luescher, U.A., McKeown, D.B. & Halip, J. (1991). Stereotypic or obsessive-compulsive disorders in dogs and cats, *Veterinary Clinics of North America: Small Animal Practice*, 21(2), 401–413.

Martin, P. (1986). An experimental study of weaning in the domestic cat. *Behaviour*, 99(3), 221-29.

Mason, G.J., (1991a). Stereotypies: A critical review. *Animal Behaviour* 41, 1015-1037.

McVittie, R. (1978). Nursing behavior of snow leopard cubs. *Applied Animal Ethology*, 4, 159–168.

de Passillé, A. M. (2001). Sucking motivation and related problems in calves. *Applied Animal Behavior Science*, 72, 175–187

Robbins, C. T. & Moen, A. N. (1975). Milk consumption and weight gain of white-tailed deer. *Journal of Wildlife Management*, 39, 355–360.

Rushen, J. & Fraser, D. (1989). Nutritive and non-nutritive sucking and the temporal organization of the suckling behaviour of domestic piglets. *Developmental Psychobiology*, 22, 789–801.

Rushen, J. & de Pasillé, A. M. (1995). The motivation of non-nutritive sucking in calves, *Bos taurus*. *Animal Behaviour*, 49, 1503–1510.

Schneirla, T. C., Rosenblatt, J.S., & Tobach, E. (1963). Maternal behavior in the cat. In H. L. Rheingold, *Maternal Behavior in Mammals*. New York: Wiley. Pp. 122-168.

Seitz, P. F. D. (1959). Infantile experience and adult behavior in animal subjects. *Psychosomatic Medicine*, 21(5), 353–378. doi: 10.1097/00006842-195909000-00002

Snook, S. S., & Riedesel, E. A. (1987). Feline Neonatal Medicine. *Iowa State University Veterinarian*, 49(2), 106-111.

Serman, M.B., Knauss, T., Lehmann, D. & Clemente, C.D. (1965). Circadian sleep and waking patterns in the laboratory cat. *Electroencephalography Clinical Neurophysiology*, 19, 509–517.

Stern, E., Parmelee, A.H. & Harris, M.A. (1973). Sleep state periodicity in prematures and young infants. *Developmental Psychobiology*, 6(4), 357-65.

Tyler, S. J. (1972). The behaviour and social organisation of the New Forest ponies. *Animal Behaviour Monographs*, 5, 85–344.

Veissier, I., de Passille, A.M., Després, G., Rushen, G., Charpentier, I., Ramirez de la Fe, A.R. & Pradel, P. (2002). Does Nutritive and non-nutritive sucking reduce other oral behaviors and stimulate rest in calves?, *Journal of Animal Science*, 80, 2574–2587.

Vogel, G.W. (1975). A review of REM sleep deprivation. *Archives of General Psychiatry*, 32(6), 749–761.

Widowski, T. M., Torrey, S., Bench, C. J., & Gonyou, H.W. (2008). Development of ingestive behaviour and the relationship to belly nosing in early-weaned piglets. *Applied Animal Behaviour Science*, 110(1-2), 109-127. doi:10.1016/j.applanim.2007.04.010

Widowski, T.M., Yuan, Y., Gardner, J.M. (2005). Effect of accommodating sucking and nosing on the behaviour of artificially reared piglets. *Laboratory Animals*, 39, 240–250.

Probing the Hydrogen Bond Network of FUS Fibril Assemblies

Rebecca Rafique*

Mentor: Dr. Dylan T. Murray
Department of Chemistry at UC Davis

Introduction

The majority of eukaryotic proteins consist of both structured and intrinsically disordered regions (IDRs) (Dunker et al., 2013). The structured regions adopt well-defined tertiary structures and have well-defined functions. The intrinsically disordered regions, on the other hand, do not have a well-defined structure yet are still important for protein functions including regulation of transcription and translation, cellular signal transduction and the ordered assembly of macromolecular machines (Dyson et al., 2005). An important class of IDRs are the low complexity domains (LCD) of RNA-binding proteins that help regulate many stages of RNA processing (Calabretta, Richard, 2015). These domains are highly biased toward a small subset of the twenty naturally occurring amino acids. These regions do not contain a large fraction of small aliphatic and bulky hydrophobic residues like most globular proteins, but consist mainly of charged and polar amino acids.

IDRs are also present in yeast prion proteins. In prion diseases, normally well folded proteins adopt a detrimental conformation. An IDR in the prion protein catalyzes the transition from a functional state to the misfolded state observed in prion diseases. Spongiform Encephalopathy,

famously known as mad cow disease, is one of the many diseases caused by these infectious prion proteins. Prion-like domains are predicted to be found in around 250 human proteins, the majority being found in DNA and RNA binding proteins (Kim, et al., 2013). Several RNA binding proteins containing prion-like domains enriched in uncharged polar amino acids are associated with neurodegenerative diseases (Harrison, et al., 2017). Prion-like domains are genetically linked to many protein misfolding diseases and result in the formation of proteinaceous aggregates in patient tissues.

Fused in sarcoma (FUS) is an RNA binding protein involved in transcription and DNA repair that contains a prion-like domain. The prion-like domain of FUS is a LCD with an abundance of Tyrosine, Glycine, Serine and Glutamine residues. During cell stress, this domain promotes the formation of membraneless organelles called RNA granules. RNA granules have properties similar to liquid droplets which are able to effectively concentrate the FUS protein in a dynamic phase. Mutant FUS lacking the N-terminal prion-like domain fails to form liquid droplets, confirming that the prion-like domain is essential for forming phase-separated liquid droplets (Patel, et al., 2015).

Genetic mutations in the primary amino acid sequence of FUS causes the liquid droplet assemblies to transition to a less dynamic gel-like phase, composed of fibrils of the FUS protein. The fibril structures are more ordered than the RNA granules and are similar to the protein fibrils of Tau and Amyloid Beta that make up the amyloids observed in Alzheimer's. The fibrils are thought to represent the pathogenic aggregated state of FUS found in the cells of patients afflicted with Amyotrophic Lateral Sclerosis (ALS) (Kwiatkoswki, et al. 2009). Mutations in FUS play a large role in the formation of these pathogenic inclusions. Comparing the liquid droplet formation by wild

type FUS and FUS containing a G156E mutation using fluorescence microscopy reveals that the mutation makes FUS less dynamic in the droplets. After 8 hours, wild type FUS remains in liquid droplet form. For the mutated FUS protein, the droplets had transitioned into more rigid fibril structures. A solution containing wild type FUS liquid droplets will eventually convert into fibrous structures: mutations such as G156E greatly accelerate the rate of fibril conversion. Recent work by solid state nuclear magnetic resonance revealed an atomic resolution structure for fibrils formed by the FUS LCD (Protein Data Bank (PDB) code 5W3N; Murray, et al., 2017). This structure suggests that interactions between polar amino acids play a significant role in the stabilization of the FUS fibrils, but this has not been investigated experimentally.

Based on the FUS fibril structure, we hypothesize that there is an intramolecular hydrogen bond network that stabilizes FUS in fibril form. To investigate this, we recombinantly expressed and purified wild type FUS-LCD and used this material to prepare fibrils with the intent of probing the thermodynamic stability of this structure using calorimetry and a urea denaturation assay. PCR site directed mutagenesis will allow us to implement specific mutations in the primary amino acid sequence designed to disrupt the potential hydrogen bond network in the fibrils. We can then quantitatively determine the thermodynamic effect of the mutations, which will help explain why the protein is prone to fibril formation. Our work is a starting point for understanding ALS-linked mutations in the FUS protein that favor the formation of fibrils.

Methods

FUS Expression

The phis FUS expression plasmid was transformed into Invitrogen BL21(DE3) cells. 2 μ l of the plasmid DNA was

incubated with 50 μ l of cells on ice for 15 min. The cells were heat shocked at 42 °C for 60 s and then incubated on ice for 2 min, 500 μ L of Luria-Broth (LB) media was added, and the cells were incubated at 37 °C with 220 RPM shaking for 30 min. 25 μ l and 100 μ l of the cells were spread onto LB/agar plates containing 100 μ g/ml ampicillin and placed in the incubator overnight. The next afternoon, a 50 ml LB culture with 100 μ g/ml ampicillin was inoculated from a single colony on the plate and incubated overnight at 37 °C with 220 RPM shaking.

A 10-fold dilution of the overnight was prepared with LB media. OD₆₀₀ values measured with a 1 cm pathlength was 6.62. 7.5 μ l of the overnight culture was added to 1 L of LB media containing 100 μ g/ml ampicillin in a 4 L baffled flask. The 1 L culture was incubated at 37 °C with 210 RPM shaking. 3 hr later, the OD₆₀₀ measured on an undiluted sample of the culture at a path-length of 1 cm was 0.676. Expression of the FUS-LCD protein was initiated by adding 0.5 mM isopropyl β -d-1-thiogalactopyranoside (IPTG), and the culture was incubated at 37 °C with 210 RPM shaking for three hr. The OD₆₀₀ measured on a 10-fold LB media dilution sample of the culture at 1 cm path-length was 2.96. The cells were harvested by centrifugation at 6,000 g for 15 min. The cell pellet was split in half, scraped into two 50 ml tubes and stored at – 80 °C.

FUS Purification (Ni²⁺ Affinity Chromatography)

The purification of the FUS-LCD utilized three different buffers: Equilibrium buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 500 mM NaCl, 6 M urea), Wash buffer (20 mM HEPES, 500 mM NaCl, 20 mM imidazole, 6 M urea) and Elution buffer (20 mM HEPES, 500 mM NaCl, 200 mM imidazole, 6 M urea).

The cell pellet was removed from the – 80 °C freezer and resuspended in 30 ml lysis buffer (6 M guanidinium hydrochloride, 50 mM Tris pH 7.5, 500 mM sodium chloride, 1% v/v Triton X- 100) with 0.25 mg/ml hen egg white lysozyme. Three tablets of Thermo Scientific Pierce Protease Inhibitor, EDTA-Free were added to the lysis solution and the mixture was tip sonicated using a Branson Sonifier 250 with a ¼ inch micro tip for 0.3 s at an amplitude of 30% with 3.0 s rest between pulses for a total on time of 1 min. The lysed cells were centrifuged at 75,600 RCF for 25 min.

The supernatant was loaded onto a Bio-Rad 5 ml Bio-scale Mini Nuvia IMAC Ni Charged column and equilibrated in equilibration buffer at a flow rate of 2 ml/min. The column was washed with 50 ml of equilibration buffer followed by 150 ml of wash buffer at a flow rate of 2 ml/min, each time until the A₂₈₀ absorbance returned to the baseline value. The protein was eluted with a gradient from 100% wash buffer to 100% elution buffer at a flow rate of 1 ml/min followed by 20 ml of 100% elution buffer.

SDS-PAGE gel samples were made from all fractions of the purification using 4x SDS loading Buffer. The volume of each fraction added to the gel sample was normalized to the wash buffer fraction volume. The gel samples were heated for 10 min at 90 °C and centrifuged before loading onto the SDS PAGE gel.

The SDS PAGE gel had a 12% resolving gel and 4% stacking gel. The gel was run in 1X SDS buffer (1:9 dilution of 10x tris-glycine-SDS running buffer with ultra-pure water) at 80 V for 15 min and 35 min at 200 V. The gel was stained using Coomassie Blue G-250 methanol-acetic acid stain and imaged using a Bio-Rad Chemi-Doc MP Imaging System. A Fisher

BioReagents EZ-Run Rec Protein Ladder was used for a molecular weight marker.

FUS Size Exclusion Chromatography

A 24 ml Bio-Rad SEC 650 column was used for size exclusion chromatography. The column was equilibrated and run using filtered and degassed buffer containing 20 mM HEPES, 500 mM sodium chloride and 6 M urea. 0.4 μ l of the 2.66 mg/ml elution sample was injected. The column was run at a flowrate of 1.0 ml/ min and 2 ml fractions were collected. SDS-PAGE gel samples were made from wash fractions exhibiting non-zero absorbance at 280 nm. The remaining procedure for the SDS-PAGE gel was the same as used for the Ni²⁺ affinity chromatography.

Fibril Formation and Imaging

3 ml of purified FUS-LCD protein at a concentration of 2.52 mg/ml was dialyzed against 20 mM HEPES buffer. 1.4 ml was recovered from dialysis and mixed with a 200 μ l solution containing 0.26 mg FUS-LCD fibril seeds. The seeds were prepared by tip sonication with a 1/8th inch microtip using a Branson Sonifier 250 operated at an amplitude of 10% with 0.1 s on time and 1 s off time for a total of 10 min. The purified FUS-LCD protein and seeds were incubated on the bench top for two days.

The 5 μ l of the FUS-LCD sequence solution was deposited on a glow discharged 200 mesh carbon copper grid and incubated for 2 min, followed by two 5 μ l washes with water for 10 s and staining with 5 μ l of 3% uranyl acetate. A JEOL JEM-1230 electron microscope was used to record negatively stained TEM images of the FUS fibrils.

PCR Site Directed Mutagenesis

The PCR reactions contained: 30 μ l autoclaved H₂O, 1.5 μ l DMSO, 2 μ l FUS template DNA (87 ng/ μ l), 1 μ l of 10 mM dNTPs, 2.5 μ l of 10 μ M forward and reverse T47A primer (sequence provided in Fig. 1 of Supplemental Information), 10 μ l of 5x Phusion GC Buffer and 0.5 μ l Phusion DNA Polymerase. The protocol on the thermocycler was one cycle of 98 °C for 30 s, one cycle of 98 °C for 7 s, one cycle of 70 °C for 20 s, then 31 cycles of 72 °C for 2 min 42 s, followed by one cycle of 72 °C for 8 min. The reaction was then kept at 4 °C overnight. The next day, 1 μ l of the New England Bio-Lab (NEB) DpnI enzyme was added to the reaction tube and incubated for 1 hr at 37 °C. The PCR product was transformed into NEB DH5 α chemically competent *E. coli* cells. 4 μ l of the PCR product was incubated with 50 μ l of cells on ice for 30 min. The cells were heat shocked at 42 °C for 30 s and then incubated on ice for 5 min, 950 μ l of SOC media was added, and the cells were incubated at 37 °C with 250 RPM shaking for 1 hr. Next, two 10-fold serial dilutions in SOC media were performed. 100 μ l of the neat and diluted cells were spread onto their respective LB/agar plates containing 100 μ g/ml ampicillin and placed in the incubator overnight. Overnights of the colonies were purified using a QIAprep Spin miniprep kit. The purified plasmid was sent to GeneWiz for sequencing.

To troubleshoot the PCR reaction, the conditions for the PCR reaction were altered. Three different conditions were tested. For these tests, the FUS template DNA (87 ng/ μ l) volume was reduced to 1 μ l. The first condition increased the DMSO volume to 3 μ l. The second condition used the Phusion HF Buffer. The third condition used 2.5 μ l of .5mM magnesium chloride. The thermocycler protocol followed a similar protocol to the previous one but replaced the 1 cycle of 70 °C for 20 s with one cycle of 68 °C for 20 s.

Three more PCR conditions were tested, each with a different starting amount of the FUS plasmid template (171 ng/ μ l). Each PCR reaction contained 30 μ l autoclaved H₂O, 1.5 μ l DMSO, 1 μ l of 10 mM dNTPs, 2.5 μ l of 10 μ M forward and reverse T47A primer, 10 μ l of 5x Phusion GC Buffer and 0.5 μ l Phusion DNA Polymerase. The amounts of FUS Template DNA varied from 2 μ l, 4 μ l and 6 μ l. The thermocycler conditions followed the same protocol as the first one but the 31 cycles of 72 °C for 2 min 42 s were changed to 31 cycles of 72 °C for 3 min.

Results

Expression and Purification of FUS

The *E. coli* recombinant protein expression system produced milligram quantities of highly pure wild type FUS protein with an N-terminal 6X His tag, having the amino acid sequence shown in Fig. 1A. The chromatogram from the single-step nickel affinity purification procedure is shown in Fig. 1B. The His-tagged FUS protein has a strong affinity for the Ni²⁺ resin column. The imidazole used in the purification buffer out-competes the FUS protein for binding to Ni²⁺ resin. A moderate 20 mM imidazole was sufficient to remove most impurities. Elution with an imidazole gradient from 20mM to 200mM revealed a single peak, suggesting a single molecular species interacts strongly with the Ni²⁺ resin. The SDS PAGE analysis of the purification fractions shown in Fig. 1C shows a strong band near a molecular weight of 70 kDa. There are faint bands consistent with molecular weights of 45 and 30 kDa also present in the elution fractions. Our result is consistent with a previous study of this FUS construct (Murray, et al., 2017). Our procedure yields 5.5 mg of His-tagged FUS-LCD from 3.9 g of wet *E. coli* cells at a purity greater than 90%.

```

ASNDYTQQA  TQSYGAYPTQ  PGQGYSSQSS  QPYGQQSSYSG  YSQSTDTSGY  GQSSYSSSYGQ  60
SQNTGYGTQS  TPQGYGSTGG  YGSSQSSQSS  YGQQSSYPGY  GQQPAPSSTS  GSYGSSSQSS  120
SYGQPQSSSY  SQQPSYGGGQ  QSYGQQQSYN  PPQGYGQQNQ  YNSSSGGGGG  GGGGGNYGQD  180
QSSMSGGGS  GGGYGNQDQS  GGGGSGGYGQ  QDRQ  214

```

Figure 1A. Residues 2-214 of the human FUS-LCD sequence. The FUS protein used in experiments contains N-terminal His-tag with the sequence MSYYHHHHHHHDYDIPTTENLYFQGAMPD.

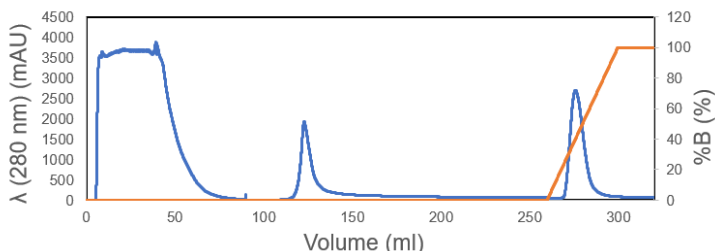


Figure 1B. The chromatogram obtained from the Ni²⁺ affinity chromatography purification of the FUS protein. The blue line is the A₂₈₀ trace and the orange line is the percent buffer B.

The first and most broad peak in Figure 1B is the flow-through from the column loading, or the proteins that did not bind to the column. The second peak is the impurities that are washed out with 20 mM imidazole, or the proteins that bind to the column with moderate affinity. The third peak is the elution fractions, or the proteins that interact strongly with the column. Gel samples were prepared for the flow-through, wash, and elution fractions with significant absorbance at 280 nm.

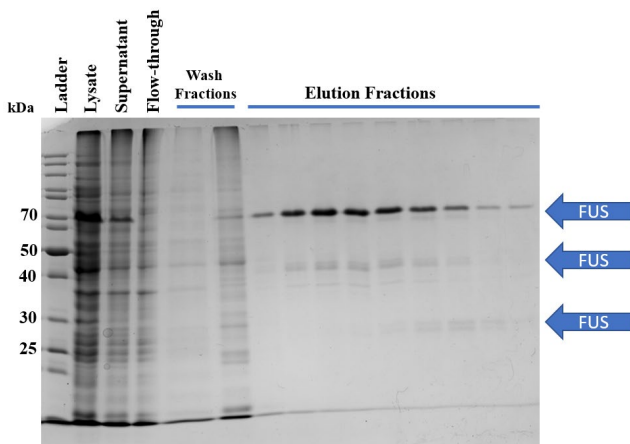


Figure 1C. The SDS PAGE gel of the fractions collected from the Ni²⁺ Affinity Chromatography.

The FUS protein has a molecular weight of 25 kDa. For the elution fractions, the most prominent bands are observed around 75 kDa, 50 kDa and 25 kDa, indicated by the 3 blue arrows in Figure 1C. The SDS-PAGE gel indicates that the FUS protein in the elution fractions exists in trimer, dimer and monomeric forms.

A gel filtration, or size exclusion chromatography (SEC) column was run on the FUS protein from the Ni²⁺ purification. The SEC chromatogram is shown in Fig. 2A. There is a single, relatively narrow, peak in the chromatogram. The location of this peak is consistent with a molecular weight species of approximately 50-100 kDa based on the manufacturer's documentation. There is no evidence of multiple molecular weight species in the chromatogram. The fractions from this peak were used to prepare gel samples for an SDS PAGE. The bands observed in the SDS PAGE gel (Fig. 2B) are similar to the 75kDa and 50 kDa bands observed in the elution fractions in the gel in Fig. 1C.

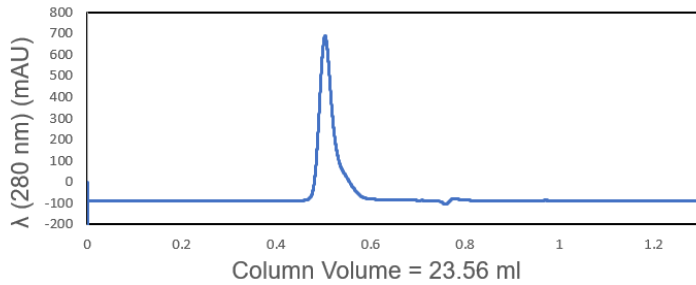


Figure 2A. The chromatogram from the size exclusion chromatography of the Ni²⁺ purified FUS protein. The blue line is the A₂₈₀ trace.

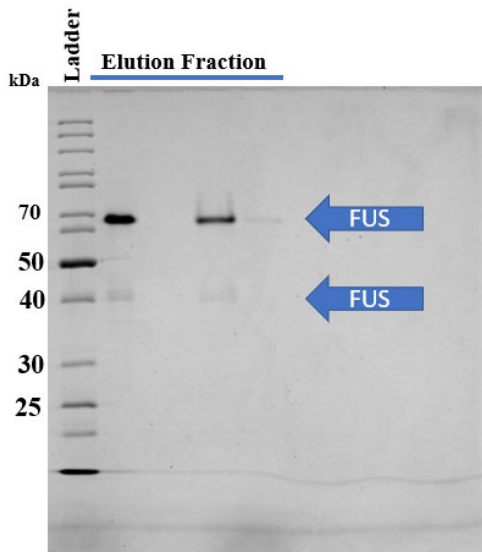


Figure 2B. An SDS PAGE gel of the size exclusion chromatography of the FUS-LCD protein.

Fibril Formation by FUS

A seeding procedure was used to form fibrils of the FUS protein. Elution fractions 2, 3 and 4 were used to create the fibrils. Urea was removed from the fractions by dialysis. Fibril seeds were prepared by tip sonicating preformed FUS fibrils and mixed with the dialyzed proteins. After 2 days, straight, unbranched fibrils formed. A transmission electron microscope (TEM) image of negatively stained FUS-LCD fibrils is shown in Fig. 3 and is consistent with previously published images of FUS fibrils (Murray, et al., 2017).

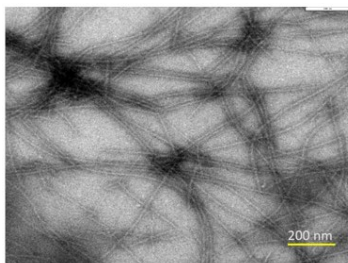


Figure 3. Negatively stained TEM image of FUS fibrils obtained at 25,000 magnification.

PCR Site Directed Mutagenesis on FUS

Residues Serine 77, Threonine 47, Serine 48 and Threonine 71 were identified as possible hydrogen bond pairs in the published structure of the FUS fibrils (Fig. 4A, PDB code 5W3N). PCR conditions for the site directed mutagenesis procedure were difficult to optimize. Most PCR reactions returned the nucleotide sequence corresponding to the wild type protein sequence shown in Fig. 1A. PCR reactions that did produce the desired mutation resulted in mis-alignment of the remaining amino acid sequence of the FUS-LCD sequence (Fig. 4B).

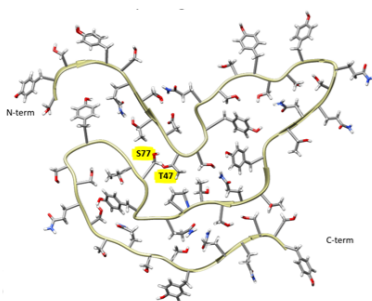


Figure 4A. The repeating units in the FUS LCD fibril structure (PDB code: 5W3N). The fibril core contains many polar residues in close proximity capable of hydrogen bonding. The putative pair of S77 and T47 are highlighted. The image was created with the UCSF Chimera program.

```
PASNDYTQQA TQSYGAYPTQ PGQGYSQQSS QPYGQQSYSG YSQSTDASGY GPEVHGRFRLC 60
QSTDASGYQS TDASGYGQST DASGYGQSTD ASGYGQSTDA SGYGQSTDA GVGHRFRLWP 120
SPRTLQAMAQ STDASGYGQS TDXSGYGQSS YSSYGQSQNT GYGTQSTPQG YGSTGG 176
```

Figure 4B. Sequencing results of the FUS-LCD sequence PCR product with the desired T47A mutation, highlighted in yellow and a misalignment of the remaining sequence starting at the underlined residue 52.

Discussion

In this paper, we have shown that 5.5 mg of pure His-tagged FUS was produced from our *E. coli* expression system and Ni²⁺ affinity chromatography purification protocol. This was confirmed through an SDS PAGE analysis. The FUS protein shows up in the SDS-PAGE gel at molecular weights of approximately 75, 50 and 25 kDa, suggesting that the FUS-LCD protein is capable of aggregation or assembly even in 6M urea. A SEC analysis confirmed that the elution fractions contained pure FUS in several oligomeric states. The protein obtained from our procedure was used to prepare fibrils that gave rise to TEM images similar to published TEM images of the FUS-LCD.

The bands at 75 and 50 kDa in the SDS PAGE gels for both the Ni²⁺ affinity chromatography and the SEC suggest that the FUS-LCD can aggregate or self-assemble in urea. These bands are consistent with trimer and dimer assemblies of the FUS-LCD. It is surprising that an intrinsically disordered protein like

the FUS-LCD still assembles into well-defined oligomeric states in a strong denaturing agent. The lack of higher order oligomeric states indicates that this assembly is well-defined and not amorphous aggregation of the protein.

The FUS-LCD is most devoid of hydrophobic residues. In the context of the protein transitioning from a state of intrinsic disorder to a well folded-protein fibril, the absence of hydrophobic residues suggests a novel balance of forces that rely mainly on hydrogen bonding interactions. How urea interferes with this process warrants further investigation. PCR site directed mutagenesis was used to introduce T47A mutation intended to disrupt a putative hydrogen bond in the FUS-LCD fibril core. Our efforts led to the T47A mutation in the primary amino acid sequence of FUS-LCD, but there was a misalignment of the remaining amino acids in the sequence. This was most likely due to the high GC content and repetitive sequences of the FUS-LCD. Alternative methods of site directed mutagenesis or a more comprehensive screen of primers and PCR conditions are required to affect mutations in the FUS-LCD sequence.

Our future efforts will quantitatively probe the thermodynamic stability of the FUS-LCD fibrils using calorimetry and a urea denaturation assay. Additional efforts will focus on obtaining mutations in the primary amino acid sequence of the FUS-LCD. Together, these efforts will elucidate the role of hydrogen bond networks in the fibril formation of the FUS-LCD protein. These measurements will then be extended to understand how ALS-linked mutations in the FUS-LCD favor the formation of pathogenic FUS fibrils.

Supplemental Information

5' – CAG TCC ACG GAC **ACT** TCA GGC TAT GGC₅₄₃₈ – 3'

5' – GCC ATA GCC TGA **AGC** GTC CGT GGA CTG – 3'

5' – GCC AGT CCA CGG **ACG** CTT CAG GCT ATG – 3'

Supplemental Figure 1. The first sequence is the template of FUS-LCD with nucleotide bases 5412-5438. The 2nd sequence is the T47A forward primer and the 3rd sequence is the T47A reverse primer. Each primer has one base modification (in red).

As shown in Supplemental Fig. 1, the codon ACT in the FUS-LCD codes for Threonine. The T47A forward primer is complementary to the FUS-LCD forward template (5'-3') with one base modification shown in red. The reverse primer is complementary to the FUS-LCD reverse template (3'-5') with one base modification. The base modification codes for Alanine.

References

- (1) Calabretta, S.; Richard, S. Emerging Roles of Disordered Sequences in RNA-Binding Proteins. *Trends in Biochemical Sciences*. 2015. <https://doi.org/10.1016/j.tibs.2015.08.012>.
- (2) Dunker, A. K.; Babu, M. M.; Barbar, E.; Blackledge, M.; Bondos, S. E.; Dosztányi, Z.; Dyson, H. J.; Forman-Kay, J.; Fuxreiter, M.; Gsponer, J.; et al. What's in a Name? Why These Proteins Are Intrinsically Disordered. *Intrinsically Disord. Proteins* **2013**. <https://doi.org/10.4161/idp.24157>.
- (3) Dyson, H. J.; Wright, P. E. Intrinsically Unstructured Proteins and Their Functions. *Nature Reviews Molecular Cell Biology*. 2005. <https://doi.org/10.1038/nrm1589>.

- (4) Harrison, A. F.; Shorter, J. RNA-Binding Proteins with Prion-like Domains in Health and Disease. *Biochemical Journal*. 2017. <https://doi.org/10.1042/BCJ20160499>.
- (5) Kim, H. J.; Kim, N. C.; Wang, Y. D.; Scarborough, E. A.; Moore, J.; Diaz, Z.; MacLea, K. S.; Freibaum, B.; Li, S.; Molliex, A.; et al. Mutations in Prion-like Domains in HnRNPA2B1 and HnRNPA1 Cause Multisystem Proteinopathy and ALS. *Nature* **2013**. <https://doi.org/10.1038/nature11922>.
- (6) Kwiatkowski TJ Jr1, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, B. R. J. Mutations in the FUS/TLS Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis. *Science (80-.).* 323 (5918), 1205–1208.
- (7) Murray, D. T.; Kato, M.; Lin, Y.; Thurber, K. R.; Hung, I.; McKnight, S. L.; Tycko, R. Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains. *Cell* **2017**. <https://doi.org/10.1016/j.cell.2017.08.048>.
- (8) Patel, A.; Lee, H. O.; Jawerth, L.; Maharana, S.; Jahnle, M.; Hein, M. Y.; Stoykov, S.; Mahamid, J.; Saha, S.; Franzmann, T. M.; et al. A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. *Cell* **2015**. <https://doi.org/10.1016/j.cell.2015.07.047>.

A case study examining the relation between community health assessments and primary health access for rural communities: Knights Landing, California

Sarina Rodriguez¹

Mentors: Dr. Natalia Deeb-Sossa^{1,2}, Jacklyn Skye Kelty², Alfonso A. Aranda²,
Department of Chicana/o Studies¹ and Center for Health and the Environment², UC Davis

Keywords: rural health, access to healthcare, community health assessments, program planning and evaluation, health access disparities, health strategy assessment

Abstract

Normalized standards for community health assessments are currently developing post-ACA, including models that highlight region-specific rural healthcare needs. This study compared various local community health assessment methodologies and described their collective influence on primary healthcare access. Knights Landing, California was a case study for unincorporated agricultural-rural community representation in health assessments and consequent health intervention strategizing. Five expert interviews were conducted with health agency representatives operating in Yolo County. Methodology and intervention development was analyzed through qualitative review of recent assessments conducted in Yolo County and subsequent responsive documentation. A preference for cost-effective health

initiatives and a lack of community participation in decision-making processes were identified causes of lowered prioritization of smaller community health needs. Local decision-making at all institutional levels was preferential to initiatives that was most cost-effective. Volatile state and federal policies changed the availability of funds for special programs customized to serve vulnerable populations in smaller communities. The Knights Landing case study demonstrated that the health needs of unincorporated communities were not reflected nor prioritized in local health assessments. Deeper examination is needed to identify subpopulations lacking adequate primary care services and to develop potential cost-effective solutions for Knights Landing and other small rural communities.

Background

Community Health Assessment Tool

Community health assessments are analytical tools used by health institutions in the U.S. and abroad to measure the needs of communities, populations, and regions via quantitative and qualitative data.¹³ In general, health institutions implement these tools to empirically identify and prioritize the needs of communities for effective planning and intervention strategies (see Figure 1). Likewise, health institutions often rely on previously published assessment goals to evaluate the principal objective of reducing local health needs.¹³ Figure 1 demonstrates a generalized interpretation of the community health assessment process as described by J. Wright, R. Williams, and J.R. Wilkinson (1998).

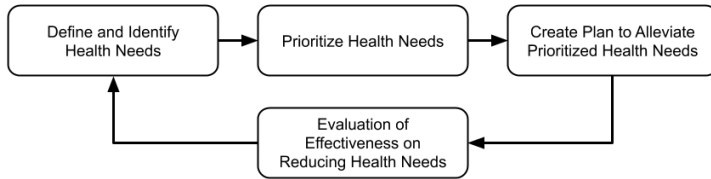


Figure 1: The cyclical process of doing community health assessments over time to define and identify the health needs of a designated region, prioritize those needs based on empirical evidence from community-collected data, and devise a plan to effectively address the highest-priority unmet health needs of said community (Wright, Williams, and Wilkinson, 1998).

Community health assessments are routine and often mandatory for many public health institutions and local governments. Most notably, the 2010 Patient Protection and Affordable Care Act (ACA) requires every not-for-profit hospital to publish a community health assessment every three years to identify and prioritize the significant health needs of the community it serves. Community health assessments currently lack a standardized model of operation which, when amplified by poor data collection, impacts accuracy of results for both rural and urban communities.¹ Current research demonstrates the need for a more objective methodology to be used for communities of varying sizes and compositions, especially to reflect rural-specific health needs accurately.^{3,8}

Rural Access to Primary Healthcare

Access to primary healthcare has been demonstrated to reduce the overall negative health outcomes of communities in the U.S.,¹⁰ yet a disparity exists between rural and urban residents and their respective levels of access.⁶ Primary healthcare access disparities are magnified when intersected with socioeconomic factors such as income, educational attainment, or race/ethnicity.² Important barriers for rural residents include provider shortages, transportation, and health center availability.^{10,12}

Despite the expansion of the ACA, significant barriers to rural health access persist at the state and national levels.⁴ Moreover, U.S. studies of rural health tend to overgeneralize the definition of rurality, leading to unreasonable conclusions about rural communities.⁴ Without a clear definition of a ‘rural community’ (or subtypes of rural communities), the analysis of rural health remains inconclusive at the expense of millions of rural Americans lacking adequate health access. Even in Canada and Australia – nations with well-established universal healthcare – achieving health access equality for rural communities is still a challenge.^{5,9} As such, rural health access remains an internationally-recognized problem for countries across diverse healthcare systems and levels of economic development.

California is no exception. For the agricultural County of Yolo in Northern California, sixteen unincorporated communities differ substantially from incorporated cities in that the former lack municipal services and rely on the county for infrastructure and capital investment. To assess the health needs of urban and rural communities in Yolo County, a number of local government agencies and hospitals have launched community health assessments. This study analyzes the diverse methodologies used in community health assessments conducted in Yolo County and the impact of these assessments on primary healthcare access for residents of rural, unincorporated communities like Knights Landing, CA.

Methods

Knights Landing, CA was selected as case study community given its unincorporated status, low population density, occupational and linguistic attributes, and the lack of public investment for healthcare reported in the vicinity. Of the five assessments published electronically in Yolo County between

2014-2019, qualitative analysis considered those conducted by the following health institutions: Yolo County Public Health Department, Dignity Health Woodland Memorial Hospital, and Sutter Davis. In effect, all three institutions examined a region that completely or almost completely represents Yolo County and included the Knights Landing zip code. Community health assessments conducted by Yolo area hospitals including Kaiser Permanente, UC Davis Health Centers, and Sacramento VA Medical Center were not included in this study either because their assessment tool was not readily available online or due to non-response after initial contact request for an interview (see below). In the case of all three county-level health assessments under review, documentation produced in response to each assessment was also included as part of secondary data analysis (e.g., community health implementation reports and community health budgets).

Although each assessment described the decision-making process behind the prioritization of health needs, reports failed to account for specific decision-making processes that determine resource allocation for health services. Thus, primary data was collected in the form of five expert interviews with representatives of Yolo-area health agencies responsible for implementing community health assessments. Participants were recruited following snowball sampling after initial contact. In this study, a ‘health expert’ was defined as anyone who holds (or has held) a volunteer or employee decision-making position at a healthcare organization serving Yolo County. All health experts interviewed either worked directly on a community health assessment in Yolo County or used community health assessments in their work.

Each interview consisted of six open-ended questions with follow-up questions. Interview questions examined the participating agency’s methodology for producing and

analyzing health assessments, the process of decision-making for resource allocation at each institution, and any perceived impacts of these assessments for rural communities. Each interview was audio recorded and transcribed to ensure accuracy of data collection, and the tapes were transcribed verbatim. Thematic analysis was conducted on all interview data via the identification of recurrent themes across interviews. Analysis was primarily focused on the process used to formulate each community health assessment and strategies for receiving community input.

This study was part of a broader, student-led effort to better understand community health status and access to medical resources in unincorporated communities. All portions of this research were validated and approved by a team of community health workers (or *promotoras de salud*) from Knights Landing trained and compensated by the Knights Landing Environmental Health Project (KLEHP).

Results

Community Health Assessment Methodologies

Each health assessment analyzed in the literature review varied in its methodology, which influenced how each institution distributed resources and services. For example, Yolo County Public Health began its most recent community health assessment in 2013 with a community survey focused on a variety of health topics including community strengths, personal health information, and individual health access barriers. In total, 579 surveys were collected countywide, but concentrated mostly in cities. Once published, county officials shared the health assessment results at ‘Road Shows’ where community members were encouraged to submit feedback. The Healthy Yolo Planning Committee comprised of Yolo County health department workers and community partners used the

new assessment to decide on health topics to serve as the focus of the 2015-2020 Community Health Implementation Plan (see Figure 2). This subsequent report focused on three health topics and included a five-year outline of health interventions and resources for each topic. These topics were also used to format the County’s HHS annual budgets until the end of the 2019/2020 fiscal year. In total, the County’s 2014 Community Health Assessment and 2015-2020 Community Health Implementation Plan directly informed over \$100 million county dollars and five years of county public health spending.

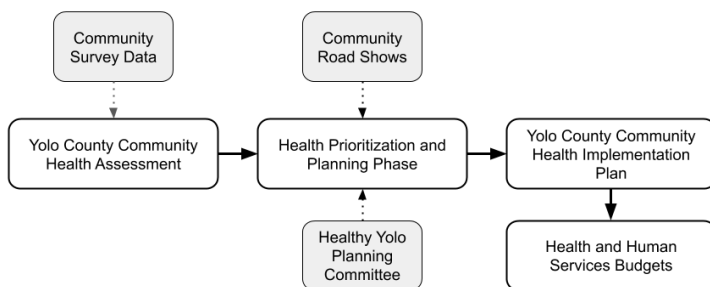


Figure 2: Community health assessment model from Figure 1 adapted to outline the 2014 Community Health Assessment process conducted by Yolo County Public Health over five years. Gray boxes with dotted arrows indicate the various points at which external input influenced the health assessment process.

Besides Yolo County Public Health, sixteen hospitals in Yolo County and the Greater Sacramento region formed a collaborative health assessment team called the Sacramento Region Collaborative Process (SRCP) in which health assessment tools and strategies were shared, but separate assessment reports were published by each respective hospital.

Dignity Health Woodland Memorial Hospital and Sutter Davis Hospital published almost identical reports in 2016 that were used over the next three years for funding and resource

allocation (see Figure 3). The SRCP health assessment process began with qualitative and quantitative data from focus groups in communities identified as being the most vulnerable. Previously published community health assessments were also reviewed with an eye for improvement. Each hospital created an algorithm to rank the eight highest priority health needs based on internal resources, mission statements, and available partnerships. These health needs were included on each hospital’s 2016-2018 Community Health Implementation Strategy reports along with suggested health initiatives. Over the next three fiscal years, Community Annual Budget Reports were released to measure the status of implementation for each outlined strategy mentioned in the Community Health Implementation Strategy. Not every hospital publicly released annual budget reports or allowed us to acquire access, but in the example of Dignity Health Woodland Memorial Hospital, the 2016 Community Health Assessment and 2016-2018 Community Health Implementation Strategy informed over \$65 million dollars of hospital spending in the Yolo County region.

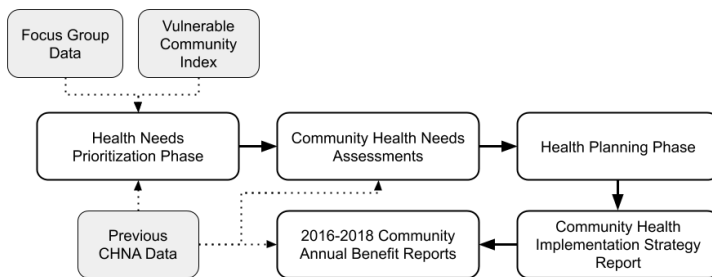


Figure 3: Community health assessment model from Figure 1 adapted to outline the 2016 Community Health Assessment process done by the Sacramento Regional Collaborative Process over three years, including Dignity Health Woodland Memorial and Sutter Davis hospitals. Gray boxes with dotted arrows indicate the various points at which external input influenced the health assessment process.

Community Participation in Community Health Assessments

Community input for the 2014 Yolo County Community Health Assessment occurred during the initial surveying period and again at ‘Road Shows’. In an effort to make the data more accessible to the public, results were also divided into seven geographical regions with a corresponding Regional Report published. Although the assessment report was made available only in English, Regional Reports were available online in English and Spanish. During the health expert interviews, interviewee(s) recommended a stronger effort on the part of local governments to engage community members to improve the public's general understandings about local health assessments and the importance of participation. In Yolo County Public Health's assessment, lowest participation occurred in rural regions including Knights Landing and its surrounding areas, severely limiting accuracy of conclusions about rural health needs. Additionally, the 2014 Community Health Assessment did not analyze any data to identify region-specific disparities, instead focusing on social influences on health such as socioeconomic and demographic health disparities. To alleviate this issue for the 2019 Community Health Assessment, Yolo County health expert interviewee(s) stated that they will deliberately implement strategies to make community data more “place-based” and better reflect region-specific disparities in health needs. Interviewee(s) suggested that Yolo County Public Health will continue to look for ways to systematically identify the health needs faced by rural towns to provide more effective rural health initiatives.

Local hospitals based much of the community input for their 2016 Community Health Assessments on focus groups targeting communities facing greater health disparities and worse health outcomes. Given the infeasibility of collecting

survey data in unincorporated towns, focus groups were designed to capture a representative sample of like-communities. Knights Landing was chosen as a ‘Focus Community’ and a focus group with nine residents was conducted in the town. For hospitals, the inclusion of small, unincorporated communities was a deliberate tactic used to identify place-based needs obscured by county-wide data. The assessment report was only published online in English. The Community Health Assessment was mostly utilized by internal employees of the hospital, some local policymakers, and community partners. Dignity Health Woodland interviewee(s) suggested further improvements for providing feedback to the communities about the results of the Community Health Assessment.

CommuniCare did not conduct a community health assessment but was an active partner in the efforts of both the County and local hospitals on their assessments. As the main Medicare provider for the county, CommuniCare made health decisions throughout the county that affect primary healthcare access. At least 51% of CommuniCare’s board consisted of patients from the community to ensure that patient needs guide health decisions. It remains difficult for CommuniCare to recruit a board member from Knights Landing mainly because of the intensity of seasonal farmwork, especially during harvest season when many patients from Knights Landing are unable to participate at board meetings. CommuniCare interviewee(s) said that they are continuing to improve communication and recruitment efforts among their patients, as well as recruitment efforts to better represent the diversity of patient and community experiences across Yolo County.

Influences Beyond the Community Health Assessment on Health Decision-Making

One of the biggest challenges faced by local health institutions – and a major emphasis in every expert interview – was the ability to economically justify the expansion of health services in small, less populous areas such as Knights Landing. Following the conclusion of each health assessment, participating institutions tended to decide on health initiatives in a fashion that created the “biggest bang for your buck”. That is, to do the most good per dollar spent. In most cases, “doing the most good” led to the prioritization of health initiatives and interventions to serve the greatest number of people. Unsurprisingly so, larger incorporated communities cities like Woodland (Pop. 60,000) and Davis (Pop. 69,000) were more likely to be recommended areas for new health services on health implementation reports, and thus were more likely to receive resources and funding to improve health primary care access.

An important point made in several interviews was that there was a higher potential efficiency of addressing access needs of small, unincorporated communities with heavier investment in upstream public health initiatives like education and infrastructure. Several top health access barriers faced in Knights Landing are considered upstream barriers and could be reduced with better transportation and language services, for example. Looking at health access as an end result of larger, systemic issues in healthcare spending, along with more funding for preventative healthcare and infrastructure in smaller communities was one theme discussed by many interviewees. Another potential tactic to address small community health needs mentioned was to make smarter partnerships with other health institutions and local non-governmental organizations that already work in small communities. Teaming up and sharing resources was described

to be one path to focusing money and human power toward targeted common goals.

Decision-making in local health institutions happened among a larger matrix of state and federal policies that limited the capacity of local health institutions to create effective region-specific health initiatives. County interviewee(s), for example, detailed many legally mandated public health responsibilities of Yolo County Public Health department beyond the community health assessment. Because the scope of their budget encompassed more than the plans mentioned in the Community Health Implementation Plan, community health spending ended up focusing on downstream “symptom management”. Financial investment into prioritized needs from the community health assessment was often downgraded compared to something that was mandated to be funded by state or federal law. Interviews with CommuniCare highlighted a similar balance of responsibilities and priorities based on federal budget mandates and policies influencing funding for projects like rural health initiatives. Swift changes in policy made to national and statewide programs often left the most vulnerable populations without reliable access to healthcare by hampering fiscal sustainability of special health services. CommuniCare, for example, could no longer justify the Knights Landing clinic staying open partially because undocumented and uninsured patients previously covered as a statewide reimbursement program no longer qualified during the Great Recession. CommuniCare shut the Knights Landing clinic down because the costs of the clinic were not reimbursed from a grant and from previously promised reimbursement aid from the state, which put the financial health of the organization at risk and drew resources from other important services.

No new health initiatives or interventions occurred in Knights Landing since the opening of the Knights Landing One Health Clinic (KLOHC). After a review of available health budgets for each institution represented in this study, \$0 have been allocated to health strategies specifically targeting the Knights Landing community and its residents. Knights Landing was included as a ‘Focus Community’ in the assessments performed by local hospitals and was included as a town on the Yolo County Public Health survey collection route and a stop on the ‘Road Shows’ where local government requested public feedback. Based on surveying completed by KLEHP in Knights Landing, however, there was a discrepancy between the top barriers to care faced by Knights Landing residents and the top prioritized health needs on local community assessments. Local community assessments did not effectively prioritize the needs of nor schedule health improvement strategies for this small, unincorporated rural town.

Discussion

Even though residents of rural unincorporated communities require healthcare, are generally covered by health insurance and pay taxes, the specific needs of these communities are poorly represented and unaccounted for in county-wide community health assessment practices. The restriction of rural voices in local community health assessments for Yolo County is partly due to current methodologies that prioritize the needs of larger population cities with lower thresholds required to overcome barriers to accessing primary health services (e.g., transportation, service provider hours of availability, etc.).

Health experts suggest improvements be made to better understand the efficacy of community health assessments at actually improving health needs. Published assessment reports have power in the local health industry, each responsible for

millions of dollars and several years of health decisions that impact millions of people in the Yolo County area. As is, the implications of a cost-focused model of health services makes it inefficient to prioritize regional health services based on reported health outcomes or health accessibility indices. But the problem goes beyond how health needs are assessed; the problem also lies in how health needs are addressed. Even health groups that inquired Knights Landing residents were not successful in bringing about any sort of change in the community. Local health experts understand the health needs of unincorporated county areas, yet find it difficult to justify the allocation of resources to these communities in a healthcare system that incentivizes economic outcomes over demonstrated health outcomes.

It is the mission of a community health assessments to represent the health needs of an entire community¹³ – this should also include rural³ and unincorporated communities. Nevertheless, despite the best efforts of local health institutions, health need prioritization seems to be determined based on choices. Whether or not a town can secure health funding becomes a matter of meeting certain demographic quotas and who gets invited to the table to make health decisions. A different system should be used to determine which rural communities get included (or not) in health needs prioritization nor health resource allocation.

Rural healthcare can be more challenging and more expensive to provide as opposed to urban healthcare.⁵ Thus, creative solutions are required to circumvent economic tensions levied when improving rural healthcare access. For Knights Landing, a non-conventional health provider like the student-run KLOHC has proven to be a more sustainable long-term operation than attempts from other providers who could not sustain the high expenses faced per patient served. Moreover,

expansion of upstream public health spending and technological advancements were mentioned by our health expert interviewees to be realistic, innovative strategies for leveling regional health disparities. Currently, the KLEHP, Yolo County health providers, and the Knights Landing community are partnering to alleviate lack of public transportation via a micro-transit system that specifically serves Knights Landing residents. New collaboration efforts across all levels of health institutions will most likely remain a goal for reducing health need disparities across the county.

One limitation to this study is that the VA Memorial Hospital in Sacramento was not included among the healthcare providers serving the Knights Landing area. Several veterans live in Knights Landing and may face other health access barriers not observed here. Additionally, the results for the newly published 2019 health assessments by both Yolo County Public Health and the SRCP were not included in this report since our interviews with health experts conducted in 2019 and our existing partnerships with their organizations may have influenced the 2016-2019 community health assessment processes. Future research should continue to take a closer examination within Knights Landing to see which subpopulations are most affected by a lack of adequate primary care services. An area of research to explore further could also investigate if similar results are rendered in other unincorporated rural communities similar to Knights Landing.

This study further demonstrates the power of community health assessments as tools for the identification and prioritization of community health needs. In Yolo County, participating institutions considered their own expertise, mission statement, and available resources to draw economically-rational goals to address the results of their assessments. Despite the multiple and simultaneous implementation of community health

assessments in Yolo County, little effort has been attempted to address the primary health service access weaknesses reported by rural unincorporated communities. As such, from the perspective of Knights Landing residents, the current process of implementation may lead to exclusion from health services planning and allocation.

Acknowledgements

This project is financially supported by UCD Jastro Fellowship, UCD George Lee Fellowship, UCD Western Center For Agricultural Health and Safety Fellowship and Rapid Response Grant (NIOSH2U54OH007550), UCD Comparative Lung Biology and Medicine T32 (HL007013), Achievement Rewards for College Scientists, and the UCD Robert E. McNair Scholars Program. Our organizational partners and mentors include the Knights Landing Family Resource Center, UC Davis Knights Landing One Health Center, Empower Yolo, and Yolo County Public Health Department. Personal thanks to the Knights Landing community, our amazing Promotoras de Salud (Ruth, Lina, Peggy, Mida, Melissa, & Paula), and the expert health interviewees. Special thanks as well to Dr. Deeb-Sossa, Jacklyn Skye Kelty, and Alfonso A. Aranda for their guidance and support.

Works Cited

1. Becker, K. L. (2014). Conducting community health needs assessments in rural communities: Lessons learned. *Health Promotion Practice, 16*(1), 15–19. doi: 10.1177/1524839914555887
2. Caldwell, J. T., Ford, C. L., Wallace, S. P., Wang, M. C., & Takahashi, L. M. (2016). Intersection of living in a rural versus urban area and race/ethnicity in explaining

access to health care in the United States. *American Journal of Public Health* 106: 1463-1469. doi: 10.2105/AJPH.2016.303212

3. Diaz, H. J.-M., Ainsworth, D., & Schmidlein, M. C. (2019). Funding priorities: data-driven approach for prioritizing community health needs in vulnerable communities. *Health Promotion Practice*, 20(4), 616–623. doi: 10.1177/1524839918771977
4. Douthit, N., Kiv, S., Dwolatzky, T., & Biswas, S. (2015). Exposing some important barriers to health care access in the rural USA. *Public Health*, 129(6), 611–620. doi: 10.1016/j.puhe.2015.04.001
5. Preston, R., Waugh, H., Larkins, S., & Taylor, J. (2010). Community participation in rural primary health care: Intervention or approach? *Australian Journal of Primary Health* 16(1): 4-16. doi: 10.1071/PY09053
6. Reschovsky, J.D., & Staiti, A.B. (2005). *Access and quality: Does rural America lag behind?* doi: 10.1377/hlthaff.24.4.1128
7. Ryan-Nicholls K. (2004). Health and sustainability of rural communities. *Rural and Remote Health* 2004, 4: 242. Retrieved from rrh.org.au/journal/article/242
8. Schuller, K. A., Cronin, C. E., Nicks, S. E., Jing, X., Kingori, C., & Morrone, M. (2019). Development and application of a rubric to compare strategies for improving access to health care in rural communities in the United States. *Evaluation and Program Planning*, 74, 61-68. doi: 10.1016/j.evalprogplan.2019.02.013
9. Sibley, L. M., & Weiner, J. P. (2011). An evaluation of access to health care services along the rural-urban continuum in Canada. *BMC Health Services Research*, 11(20). doi: 10.1186/1472-6963-11-20

10. Starfield, B., Shi, L., & Macinko, J. (2005). Contribution of primary care to health systems and health. *The Milbank Quarterly*, 83(3), 457–502. doi: 10.1111/j.1468-0009.2005.00409.x
11. Sweeney, J. M., Zielinska Crook, P., Deeb-Sossa, N., Tu, B., Dear, J. D., & Mazet, J. (2018). Clinical one health: A novel healthcare solution for underserved communities. *One Health (Amsterdam, Netherlands)*, 6, 34–36. doi: 10.1016/j.onehlt.2018.10.003
12. Syed, S. T., Gerber, B. S., & Sharp, L. K. (2013). Traveling towards disease: Transportation barriers to health care access. *Journal of Community Health*, 38(5): 976-993. doi: 10.1007/s10900-013-9681-1
13. Wright, J., Williams, R., & Wilkinson, J. R. (1998). Development and importance of health needs assessment. *BMJ*, 316: 1310. doi: 10.1136/bmj.316.7140.1310

Bilingualism and Executive Function: Emerging Bilinguals in Head Start

Diana Alicia Santoyo*

Mentor: Dr. Yuuko Uchikoshi
School of Education at UC Davis

Abstract

The aim of this study is to examine dual language learners' (DLLs) bilingual proficiency and the association between proficiency and executive function (EF) components of inhibitory control and cognitive flexibility. Data were collected from 31 Mexican-American and 53 Chinese-American preschoolers enrolled in Head Start centers in Northern California. Results show that, on average, Spanish-English and Cantonese-English DLLs score similarly low in both English and their home language. There were no significant differences between the Mexican-American and Chinese-American children on the oral language measures in both L1 and English. Furthermore, despite their low oral proficiency, EF scores suggest that on average they score similarly to their monolingual-English speaking peers. DLLs with stronger language skills in both languages tended to do better on EF than those with low skills in both languages. Implications for educational policy and classroom instruction will be discussed.

Theoretical Framework

In the last decade, dual language learners (DLLs; children who are exposed to and learning through two languages) in the United States have increased from 20 million to 23 million (or from 28% to 32% of all children). DLLs are more likely to live

under poverty and have less-educated parents compared to their monolingual English-speaking peers (Child Trends, 2016). Although the majority of DLLs are from Spanish-speaking households, most recently, there has been a dramatic increase in DLLs from Chinese-speaking households (Child Trends, 2016). Much of the past studies have been with Spanish-English DLLs; not much research has been conducted with DLLs with other language combinations, such as Chinese-English DLLs (Hammer et al., 2014). In particular, how similar or different Chinese-English and Spanish-English DLLs in Head Start programs are under-explored.

Moreover, despite research suggesting that bilingualism may be associated with some cognitive benefits, DLLs from low-income families lag significantly behind their monolingual peers on school readiness measures at kindergarten entry, and this achievement gap widens with age. Furthermore, current literature has contrasting views on whether or not bilingualism promotes children's school readiness skills, cognitive ability and socioemotional outcomes. Some researchers have found no bilingual advantage in EF in inhibitory control or set shifting (Paap, Johnson, & Sawi, 2015). Yet, others suggest that bilingualism enhances children's executive function due to their ability to inhibit one language while speaking another language (Bialystok, 2001). This is known as the bilingual advantage in executive control (Bialystok, 2001). The effects of bilingual advantage appear to be specific in some executive domains, such as inhibitory control (Bialystok & Senman, 2004; Bialystok & Shapero, 2005) and cognitive flexibility (Costa, Hernández, & Sebastián-Gallés, 2008; Prior & Macwhinney, 2010). Essentially, it is important to look into EF because it is responsible for the tasks we complete daily. The mental processes enable our capacity to plan, organize, and monitor the execution of behaviors. The development of EF

throughout childhood and onward is the influencer to behavior, mental flexibility, and self-control.

Thus, more research is needed to understand (1) the bilingual proficiency of both Spanish-English and Chinese-English DLLs, and (2) the relationships between young DLLs' bilingual proficiency and EF skills.

Research Question

The aims of this study are to examine (1) the levels of bilingual proficiency in young Chinese-American and Spanish-American children and (2) the association between language proficiency in the dominant and nondominant languages of Spanish and English and Cantonese and English and the executive function (EF) components of inhibitory control and cognitive flexibility with emerging Spanish-English and Cantonese-English bilingual preschoolers enrolled in Head Start programs in Northern California.

Methods

Participants

A total of 84 preschoolers attending Head Start programs and their parents in Northern California were recruited for this study. In total, there were 31 Spanish-speaking Mexican Americans [MA] and 53 Cantonese-speaking Chinese Americans [CA] DLLs. All children were between 36 and 60 months of age. The average age of the participants was 49.23 months. There were no differences in the average age of the MA and CA participants. Because of our focus on low-income families, the children and parents were recruited from Head Start centers in Northern California. With respect to language and literacy skills, the children were exposed to Spanish or Cantonese at home and had to be able to produce two-word

telegraphic speech in Spanish or Cantonese before age three (based on index used in other studies of DLLs). Data collection by trained research assistants speaking either native English, native Spanish or native Cantonese was done in the children's homes over a 3-hour period

Parents of the children have the following demographics. On average, they have 11 years of education with a per capita income of \$8,571. In addition, these parents have been residing in the United States on average of 9 years. There does not seem to be a significant difference between MA and CA families on parental education, socioeconomic status, and residency in the U.S.

Measures

Language Proficiency

Language proficiency was measured with the Woodcock-Johnson IV Tests of Oral Language (WJ IV OL; Schrank, Mather, & McGrew, 2014). Specifically, we used the Picture Vocabulary, Oral Comprehension, and Understanding Directions subtests in both the first language (L1; Spanish or Cantonese) and English. The Picture Vocabulary subtest (WJPV) requires the child to name both familiar and unfamiliar pictures, ordered by increasing difficulty, with each response scored as correct or incorrect by the assessor. The Oral Comprehension subtest (WJOC) requires the child to listen to and supply a missing word to the end of a sentence, or related group of sentences. The Understanding Directions subtest (WJUD) requires the child to look at a picture and respond to verbal requests to point to certain items on the picture. The English version was used to assess English oral skills. The Spanish version was used to assess Spanish oral skills. The Spanish version was translated to Cantonese and used to assess

Cantonese oral skills as done in past research (e.g. Uchikoshi, 2013).

Executive Function

EF was measured with the NIH ToolBox Dimensional Change Card Sort Test and Flanker Test and the Head-Toes-Knees-Shoulders Task.

NIH ToolBox Dimensional Change Card Sort Test (Age 3-7) (DCCS)

DCCS is a measure of cognitive flexibility -- the ability to shift attention between tasks. This test has been widely used to measure executive function in children (Zelazo et al., 2013). During the task, participants are presented two targeted cards on an iPad, and then assessed on their ability to distinguish the dimensional differences (shape or color) by selecting a series of test cards. This test has shown high test-retest reliability (ICCs=.86-.95, Zelazo et al., 2013).

NIH ToolBox Flanker Test

Flanker test is an inhibitory control and attention measures. This test was originated from the Eriksen flanker task (Eriksen & Eriksen, 1974). In the flanker test, participants are presented with 5 arrows horizontally. They are asked to indicate where the middle arrow is pointing, while inhibiting attention to the arrows next to it. The NIH Toolbox first presented fish instead of arrows, this is designed to get children's attention and engage them more with the task. Difficulty increases as the child continues and eventually, trial will no longer be presented with fish but arrows. The flanking stimuli can sometimes be congruent with the middle orientation and sometimes can be incongruent. This test has shown high test-retest reliability (ICCs=.86-.95, Zelazo et al., 2013).

Heads-Toes-Knees-Shoulders (HTKS)

In this task, children are asked to play a game in which they must do the opposite of what the experimenter says. The HTKS task has been conceptualized by Ponitz et al., (2008) as a measure of inhibitory control, working memory and attention focusing.

Statistical Analysis

Group differences between MA and CA were examined with t-tests using the Satterthwaite approximation. The means, standard deviation, and ranges of the scores are presented in Table 1. Groups were collapsed and grouped using cluster analysis and results of the cluster analysis were used to examine relationships with EF.

Results

Descriptive Analysis

The performance of MA and CA children in language and EF measures is summarized in Table 1.

Language Performance

As shown in Table 1, although the range in scores was large, on average, children scored over one standard deviation below the monolingual age-equivalent norms on the English oral proficiency measures. On the picture vocabulary task, there were no differences in English expressive vocabulary standard scores between the two groups; $t(49.42)=-.34$, $p=.7357$. Similarly on the oral comprehension task, there were no differences in standard scores between the two groups; $t(46.01)=-1.19$, $p=.2405$. This was also true for the understanding directions task, where there were no differences in the standard scores between the two groups; $t(64.1)=-.47$, $p=.6364$.

Table 1. Descriptive statistics of language measures for all children ($n = 84$), all Mexican-American children ($n = 31$), all Chinese-American children ($n = 53$).

Variables	Total Range	Total Mean (SD)	MA Children (SD)	CA Children (SD)
Eng. WJPV Range	0-24	12.06 (5.17)	12.23 (5.53)	11.96 (4.99)
Eng. WJPV SS	40-117	76.88 (16.88)	77.79 (18.73)	76.35 (15.88)
Eng. WJOC Raw	0-12	1.15 (2.14)	1.32 (1.89)	1.04 (2.29)
Eng. WJOC SS	48-118	68.95 (14.56)	71.71 (16.78)	67.33 (13.01)
Eng. WJUD Raw	0-21	7.95 (5.58)	8.40 (5.56)	7.67 (5.64)
Eng. WJUD SS	40-125	80.16 (16.93)	81.32 (15.28)	79.48 (17.95)
L1 WJPV Raw	0-22	9.91 (6.45)	10.90 (7.01)	9.31 (6.08)
Sp. WJPV SS	40-110	69.14 (24.91)	69.14 (24.91)	- -
L1 WJOC Raw	0-8	1.30 (1.93)	1.89 (2.41)	0.96 (1.49)
Sp. WJOC SS	40-94	61.07 (18.55)	61.07 (18.55)	- -
L1 WJUD Raw	0-21	4.97 (4.20)	5.37 (5.03)	4.74 (3.63)
Sp. WJUD SS	40-103	64.75 (19.84)	64.75 (19.84)	- -

Note. SS = Standardized scores. L1 includes Spanish (Sp) and Cantonese (Ca).

Of the three English oral proficiency measures, when compared to the published age-matched monolingual English population norms, on average, children did best on the understanding directions subtest. This may be due to the fact that this is a receptive measure and the child only needs to point to the answers, as opposed to the other measures that require the child to verbally respond. For the other two tasks, the children, on average, scored more than 1.5 standard deviations lower than the published age-matched English monolingual norms.

When examining the Spanish standard scores, we found that on average the children scored over one standard deviation below the monolingual age-equivalent norms. The raw scores in Spanish and Cantonese inform us that on average both groups scored similarly low on their home language measures. T-test analysis using raw scores show no differences between the MA and CA groups (L1 picture vocabulary task: $t(54.82)=-1.03$, $p=.3072$; L1 oral comprehension task: $t(42.33)=-1.70$, $p=.0958$; L1 understanding directions task: $t(47.47)=-.60$, $p=.5521$).

Executive Function Performance

The means, standard deviation, and ranges of executive function performance are presented in Table 2.

Table 2. Descriptive statistics of executive function measures for all children ($n = 84$), all Mexican-American children ($n = 31$), all Chinese-American children ($n = 53$).

Variables	Total Range	Total Mean (SD)	MA Children (SD)	CA Children (SD)
NIH Raw (Flanker)	0-40	12.66	13.75	10.48
(9.40)		(9.39)	(9.02)	
NIH Age-Corrected (Flanker)	0-122	80.13	84.96	72.24
		(35.48)	(34.40)	(43.00)
NIH Raw (DCCS)	0-36	7.81	5.63	8.00
		(9.60)	(8.62)	(11.01)
NIH Age-Corrected (DCCS)	0-115	77.37	56.29	87.66
		(36.70)	(46.53)	(26.49)
HTKS Raw	0-47	4.83	4.00	5.31
		(9.96)	(8.23)	(10.89)

When compared to monolingual age-equivalent norms on the EF tasks, on average, the children scored over 1.5 standard deviations below the published mean for both the Flanker and DCCS measures. However, the variation in scores were large. Interestingly, the MA group scored higher than the CA group on the Flanker task, $t(75.84)=-2.03$, $p=.0454$, but there were no significant group differences on the DCCS task, $t(44.02)=1.94$,

$p=.0589$, and the Head-Toes-Knees-Shoulders Task, $t(68.97)=.59$, $p=.5548$.

Cluster Analysis

As there were no significant differences between the English oral language scores and the L1 oral language scores, both MA and CA groups were merged to see if there were any differences among the combined abilities of L1 and English among the participants. Figure 1 shows the relationship between L1 picture vocabulary and English picture vocabulary by group. Figure 1 suggests that both MA and CA participants had similar varied English and L1 vocabulary knowledge.

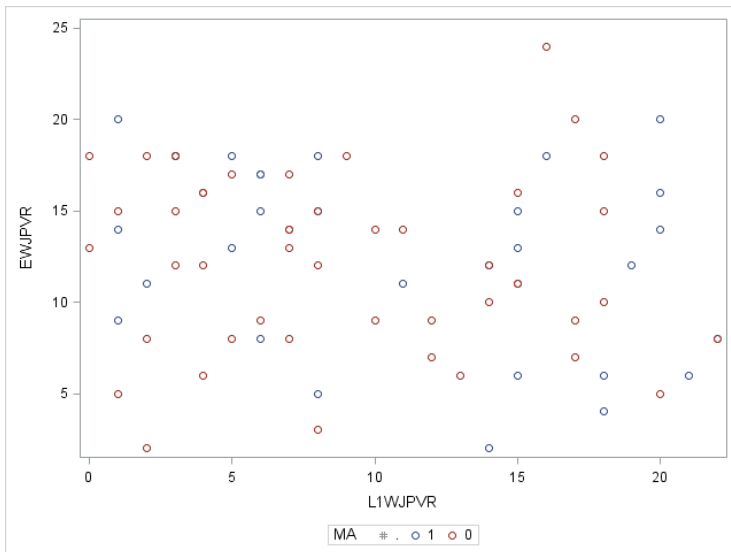


Figure 1. Relationship between English picture vocabulary (EWJPVR) and L1 picture vocabulary (L1WJPVR) by Mexican American (blue) and Chinese American (red) group.

Using agglomerative cluster analysis (Ward's method) on all subjects with raw scores on the L1 and English picture vocabulary subtest, four clusters emerged. Examining the four

clusters revealed that the largest cluster included children who had higher English vocabulary scores and low L1 scores (English dominant), the next largest had children with high L1 scores and low English scores (L1 dominant), followed by those who had low L1 and low English (low bilingual), and those with equivalently high English and L1 scores (ideal bilingual). The means, standard deviation, and ranges of the scores for vocabulary, and EF subtests by clusters are presented in Table 3.

Table 3. *Four clusters analysis.*

Variables	Ideal Bilingual (n=10)	English Dominant (n=31)	L1 Dominant (n=23)	Low Bilingual (n=12)
Eng. vocab.	17.60	15.61	8.43	6.83
Mean (SD)	(3.06)	(2.22)	(2.90)	(2.66)
L1 vocab.	17.50	5.00	15.91	4.33
Mean (SD)	(2.01)	(2.95)	(3.42)	(2.67)
NIH Age-Corrected (Flanker) Mean (SD)	96.90 (9.71)	84.07 (27.10)	92.26 (25.25)	59.00 (52.88)
NIH Age-Corrected (DCCS) Mean (SD)	93.80 (5.96)	76.00 (35.02)	75.61 (41.92)	69.67 (42.80)
HTKS Raw Mean (SD)	1.80 (4.69)	4.50 (10.11)	7.39 (11.78)	0.54 (1.29)

Executive Function

Results show a large variation on the NIH ToolBox EF tasks among the groups. Interestingly, the variation on the NIH ToolBox EF measures was the smallest for the “ideal bilingual” group.

A one-way between subjects ANOVA was conducted to compare the means of executive function tasks by clusters. There were significant differences on the Flanker task among the four cluster groups [$F(3, 72) = 3.11, p = .0314$]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the ideal bilingual group ($M = 96.9, SD = 9.71$) was significantly different than the low bilingual group ($M = 59, SD$

= 52.88). However, the other groups did not significantly differ from each other.

Although the means varied among the four groups, a one-way between subjects ANOVA revealed no significant differences on the DCCS task among the four cluster groups [$F(3, 72) = .88$ $p = .4560$]. Similarly, the HTKS task showed large variation in the means among the four groups, and a one-way between subjects ANOVA revealed no significant differences on the HTKS task among the four cluster groups [$F(3, 69) = 1.65$ $p = .1854$].

Discussion

The findings of this study suggest that at the beginning of Head Start, Spanish-English DLLs and Cantonese-English DLLs may have more similarities than differences in the language and executive function skills. On average, both sets of DLLs appear to be struggling to increase their oral proficiency in both languages. In English, on average, both groups were over 1.5 standard deviations below the published English norm. Their L1s were on average similar to their English oral proficiency. This supports past studies where DLLs performed below the monolingual English children on oral proficiency (Hammer et al., 2014). At the same time, there appears to be large variations in proficiency with some DLLs achieving bilingual proficiency, while many were language dominant in either English or their home language. This may be due to a variety of factors as shown in previous research, such as language exposure and mother's education (Hammer et al., 2014).

As for executive function, similar to past research, there appears to be differences in results based on the task. Group differences were seen in the Flanker task, yet all groups performed similarly on the DCCS and HTKS tasks. Flanker

task tests for inhibition. Our results suggest that children who have stronger language skills in both languages tend to do better with inhibitory control when compared to children who have weaker language skills in both languages. This is in line with previous research where bilingual children performed better in the inhibitory control task than monolinguals (Bialystok & Senman, 2004; Bialystok & Shapero, 2005).

Implications

One implication is that all DLLs need to increase their oral proficiency. They need to be exposed to a variety of vocabulary and have more opportunities to use their language in either or both of their languages. Past research has shown that the quality and quantity of their language exposure has an impact on children's language development (Rowe, 2012).

Another implication is that children can improve their oral proficiency in their home language without hindering their English language development. A group of DLLs were able to reach proficiency in both of their languages. It appears that if children are able to achieve some level of bilingual proficiency, this may impact their EF systems.

Limitations

The limitations of this study involve the sample size and criteria. A larger sample would give more power to this study. Since the participants came from a specific area, namely Northern California, and were from a specific population (MA and CA), these results are not generalizable to everyone who speaks more than one language. Another limitation concerns the hand dominance of the children when doing the NIH ToolBox. Children tended to forget to put their dominant hand on home base or began using their nondominant hand. This can cause a discrepancy in time calculation and scoring.

Significance

The results from this study show that the Chinese-American and Mexican-American children in this study may have more similarities than differences. This may have implications for preschool and head start classrooms. All teachers for all students need to focus on improving the children's oral proficiency, especially vocabulary, in either or both of the languages of bilingual children. Parents can also be advised to interact with their children in their home language as children proficient in both languages may have an advantage in particular areas of EF, such as inhibition. As this is one of the few studies examining young DLLs, more research is needed to further examine the relationship between bilingualism and executive function at younger ages.

References

Bialystok, E. (2001). *Bilingualism in development: Language, literacy, and cognition*. New York, NY, US: Cambridge University Press <http://dx.doi.org/10.1017/CBO9780511605963>

Bialystok, E. & Senman, L. (2004). Executive processes in appearance–reality tasks: The role of inhibition of attention and symbolic representation. *Child Development*, *75*, 562-579. doi:10.1111/j.1467-8624.2004.00693.x

Bialystok, E. & Shapero, D. (2005). Ambiguous benefits: the effect of bilingualism on reversing ambiguous figures. *Developmental Science*, *8*, 595-604. doi:10.1111/j.1467-7687.2005.00451.x

Child Trends. (2016). Dual language learners. Retrieved from <https://www.childtrends.org/indicators/dual-language-learners>

Costa, A., Hernández, M., & Sebastián-Gallés, N. (2008). Bilingualism aids conflict resolution: Evidence from the ANT

- task. *Cognition*. 106(1), 59-86.
<https://doi.org/10.1016/j.cognition.2006.12.013>.
- Eriksen, B. A. & Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & Psychophysics*, 16(1), 143–149.
<https://doi.org/10.1186/1744-9081-3-42>
- Hammer, C. S., Hoff, E., Uchikoshi, Y., Gillanders, C., Castro, D. C., & Sandilos, L.E. (2014). The language and literacy development of young dual language learners: a critical review. *Early Childhood Research Quarterly*, 29, 715-733. doi: 10.1016/j.ecresq.2014.05.008
- Paap, K. R., Johnson, H. A., & Sawi, O. (2015). Bilingual advantages in executive functioning either do not exist or are restricted to very specific and undetermined circumstances. *Cortex: A Journal Devoted to the Study of the Nervous System and Behavior*, 69, 265-278.
<http://dx.doi.org/10.1016/j.cortex.2015.04.014>
- Ponitz, C. C., McClelland, M. M., Matthews, J. S., & Morrison, F. J. (2009). A structured observation of behavioral self-regulation and its contribution to kindergarten outcomes. *Developmental Psychology*, 45(3), 605–619.
<https://doi.org/10.1037/a0015365>
- Prior, A., & Macwhinney, B. (2010). A bilingual advantage in task switching. *Bilingualism: Language and Cognition*, 13(2), 253–262. <https://doi.org/10.1017/S1366728909990526>
- Rowe, M. L. (2012). A Longitudinal Investigation of the Role of Quantity and Quality of Child-Directed Speech in Vocabulary Development. *Child Development*, 83(5), 1762-1774.
- Schrank, F. A., Mather, N., & McGrew, K. S. (2014). *Woodcock-Johnson IV Tests of Oral*

Language. Rolling Meadows, IL: Riverside.

Uchikoshi, Y. (2013). Predictors of English reading comprehension: Cantonese-speaking English language learners in the U.S. *Reading and Writing: An Interdisciplinary Journal*, 26(6), 913-939. <http://dx.doi.org/10.1007/s11145-012-9398-z>

Zelazo, P. D., Anderson, J. E., Richler, J. , Wallner-Allen, K. , Beaumont, J. L., & Weintraub, S. (2013). II. NIH ToolBox Cognition Battery (CB): Measuring executive function and attention. *Monographs Society Res Child*, 78, 16-33. doi:10.1111/mono

Fibril Formation by the TIA1 Low Complexity Sequence Domain

Rachelle Stowell*

Mentor: Dr. Dylan T. Murray
Department of Chemistry at UC Davis

Abstract

Amyotrophic lateral sclerosis and frontotemporal dementia are devastating neurodegenerative diseases that are poorly understood with no effective treatments. Mutations in the T cell-restricted intracellular antigen-1 (TIA1) protein linked to these diseases correlate with an increased incidence of pathogenic fibrillar aggregates in patient tissue, similar to the buildup of amyloid plaques observed in Alzheimer's patients. TIA1 is an RNA-binding protein that is essential for the formation of RNA stress granules, which are micron sized structures that protect cells in disease conditions. Pathogenic fibrillar assemblies of TIA1 are thought to nucleate from the RNA granule structures. In this study, we produced the TIA1 protein using a recombinant bacterial expression system, obtained milligram quantities of the protein, and have purified it to greater than 95% purity. TIA1 fibrils were prepared and transmission electron microscopy was used to visually characterize their appearance. A protein denaturation assay is employed to measure the thermodynamic stability of TIA1 in fibril form and solid state nuclear magnetic resonance to characterize the fibril structure. The aim of this study is to quantitatively characterize the mechanisms by which RNA granule formation and fibrillization occur. Results will allow for a better understanding of the TIA1 assembly in a biological context.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating disease that leaves patients with a short-lived future. Some patients with ALS also express symptoms of frontotemporal dementia (FTD). The death of motor neurons in ALS patients leads to symptoms such as weak limbs, respiratory failure, and impairment of speech. In FTD, neuronal degeneration occurs in the frontal and anterior temporal lobes and is identified through behavioral symptoms such as changes in mood, apathy, and repetitive behaviors or actions (Langenhove, Zee, & Broeckhoven, 2012). Approximately 50% of ALS patients exhibit impaired cognitive abilities (Ringholz et al., 2005). After onset and prognosis, the typical lifespan of an ALS patient with or without FTD is approximately 3-5 years (Rowland & Shneider, 2001). While ALS and FTD have been commonly distinguished as different diseases, there is evidence that these disease pathways may be linked (Ling, Polymenidou, & Cleveland, 2013). Current, clinically approved drugs only marginally increase the lifespan of a patient by 2-3 months (Renton, Chiò, & Traynor, 2014). There is currently no cure or concrete understanding of what causes these diseases.

With gene sequencing becoming more accessible, we have discerned underlying genetics of neurodegenerative diseases. Whole genome sequencing has identified six specific amino acid mutations in the T cell-restricted intracellular antigen-1 (TIA1) protein linked to ALS pathology (Mackenzie et al., 2017). TIA1 is an RNA-binding protein (RBP) that regulates alternative RNA splicing (Meyer et al., 2018). Mutations of TIA1 that are linked to ALS occur in the low complexity sequence (LCS) domain: a domain that drives the protein-protein interactions of TIA1.

RBPs with LCS domains exist in at least three states: intrinsically disordered, dynamic phase separated, and rigid fibril. These proteins can be recruited into micron sized membraneless organelles, called RNA granules. This process is a normal and healthy function of the cell. A proposed purpose of an RNA granule is to help regulate translation (Anderson & Kedersha, 2002). Little is known about the mechanisms that create RNA granules, but the LCS domains in RBPs play a significant role.

An LCS domain is a protein amino acid sequence that is highly enriched in only a few amino acid types. Four amino acids comprise 52% of the TIA1-LCS domain, which is 116 residues long. LCS-domains typically do not adopt a rigid three-dimensional structure, but are important for cellular functions such as RNA granule formation (Banani, Lee, Hyman, & Rosen, 2017). The LCS domain is necessary and sufficient for phase separation (Gilks et al., 2004; Kedersha, Gupta, Li, Miller, & Anderson, 1999; Lin et al., 2015; Mackenzie et al., 2017). Experiments that investigated the ability of different domains of RBPs with LCS domains show that the LCS domain, but not the RNA-binding domain, will form phase separations (Gilks et al., 2004; Molliex et al., 2015).

RNA granules are transient structures composed of molecules with highly fluid and dynamic properties (Buchan & Parker, 2009). A cell regulates RNA granule formation and dissociation based on extracellular conditions. RNA-binding proteins with mutations in the LCS-domain can cause RNA granules to mature, and result in proteinaceous aggregates in the cell (Murakami et al., 2015). Protein-protein phase separations of purified proteins *in vitro* reproduce the macroscopic behaviors of RNA granules (Riback et al., 2017). TIA1 phase separations mature and become less dynamic over time and mutations in the TIA-LCS domain increase the

likelihood in which this maturation occurs (Mackenzie et al., 2017). These physical changes are due to the formation of amyloid-like protein fibrils (Lin et al., 2015). These fibrils have a well-defined structure that can be studied to gain a greater understanding of the biological behaviors of the RBPs, such as TIA1.

There are few molecular studies that investigate TIA1-LCS in fibril form and how this relates to the distinct thermodynamic states in living cells. Here, we show that the TIA1-LCS can be expressed and purified using a recombinant *E. coli* expression system and that fibrils of TIA1-LCS can be reliably produced from this material. Our work lays a foundation for future structural and thermodynamic studies of TIA1-LCS in the phase separated and fibril states.

Methods

E. coli Transformation and Expression

An expression plasmid for 6x His-tagged TIA1-LCS was transformed into chemically competent BL21(DE3) *E. coli* cells using standard methods. An aliquot of the BL21(DE3) *E. coli* was thawed on ice. 2 μ l of the plasmid DNA was added to the bacteria, was gently mixed, and placed on ice for 15 min. The mixture was then heat shocked at 42 °C for 90 s. 500 μ l of Luria Broth (LB) media was added and the mixture was placed in a shaker-incubator at 37 °C, 220 rpm, for 20 min. The transformed *E. coli* were streaked onto an LB agar plate containing 100 μ g/ml ampicillin and incubated at 37 °C overnight.

For large-scale expression in LB media, a single colony was picked from the plate and was used to inoculate 50 ml of liquid LB media containing 100 μ l/ml ampicillin. The culture was placed in an incubator at 37 °C overnight with shaking at 220

RPM. The following morning, 20 ml of this culture was transferred into 1 l of LB media with 100 µg/ml ampicillin in a 4 l baffled flask. The culture was grown at 37 °C with shaking at 220 RPM until the OD₆₀₀ reached 3.32. Protein expression was induced by adding 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the culture grown at 37 °C with 220 RPM shaking for 3 hr. The cells were harvested by centrifugation at 6,000 g, flash frozen in liquid nitrogen, and stored at -80 °C.

For the expression of TIA1-LCS in minimal media (45 mM sodium phosphate dibasic heptahydrate, 8 mM sodium chloride, 20 mM potassium phosphate monobasic, 19 mM ammonium chloride, 5% w/v glucose, 2 mM magnesium chloride, 100 µM calcium chloride), an LB agar plate containing 100 µg/ml ampicillin was streaked from a -80 °C stock of the previously transformed BL21(DE3) *E. coli* using a pipette tip and incubated at 37 °C overnight. Two 50 ml LB cultures were inoculated with the bacteria from the agar plate using a pipette tip. 100 µg/ml of ampicillin was added to each culture and incubated overnight at 37 °C and 225 RPM. The next morning, the cultures were removed from the shaker and 20 ml of each culture was transferred into a separate 1 l of LB media. 100 µg/ml of ampicillin was added to each 1 l flask. The flasks were incubated at 37 °C with 225 RPM shaking. After approximately three hours, the cultures were harvested at 6,000 g for 10 min. The supernatant was discarded, the cell pellets were resuspended with approximately 10 ml of minimal media each and transferred together into 1 l of minimal media. 100 µg/ml of ampicillin was added to the minimal media and the solution was incubated at 37 °C, 225 RPM, for 30 min. The OD₆₀₀ was 1.626 a.u. measured on a 1 cm pathlength. 0.5 mM IPTG was added to the culture to induce protein expression and the culture was incubated for approximately 3 hr at 37 °C, 225 RPM. The OD₆₀₀ after three hours was 2.12 a.u. measured on a

1 cm pathlength. The culture was harvested at 6,000 g for 15 min and the cell pellet was flash frozen in liquid nitrogen and stored at -80°C until purification. The wet cell pellet weight was 2.8 g.

Purification of TIA1-LC

For the purification of TIA1-LCS expressed in LB media, the cell pellet was removed from the -80°C freezer and thawed on ice. 20 ml of lysis buffer (6 M urea, 20 mM Tris pH 7.5, 200 mM sodium chloride, 200 mM imidazole, 1% v/v Triton X-100) containing 0.028 g of powdered hen egg white lysozyme were used to resuspend the cell pellet. The cell suspension was lysed using a Branson Sonifier 250 with a 1/4" tip with the settings 1 s on, 3 s off, 30% output until the solution was no longer viscous. The solution was transferred to a 50 ml centrifuge tube, 1 ml of lysis buffer was added, and spun down at 75,600 g for 30 min at 4°C . The supernatant was harvested and loaded onto a 5 ml Bio-Rad Mini Nuvia IMAC Ni^{2+} -Charged column equilibrated in equilibration buffer (6 M urea, 20 mM Tris pH 7.5, 200 mM sodium chloride). The column was washed with equilibration buffer and then equilibration buffer with 20 mM imidazole, each time until the A_{280} absorbance returned to baseline. The protein was eluted with 200 mM imidazole. Fractions were collected from all steps of the purification and aliquots from each fraction were saved for SDS-PAGE analysis.

For the purification of TIA1-LCS expressed in minimal media, the cell pellet was removed from the -80°C freezer and was thawed on ice. 25 ml of lysis buffer containing 625 μl of hen egg white lysozyme was used to resuspend the cells using a serological pipette. Phenylmethylsulfonyl fluoride was added to the resuspension for a final concentration of 1 mM. The pellet was placed into a container of ice and water and was sonified with a Branson Sonifier 250 and 1/4" tip for 1 min

using the following settings: 0.3 s on, 3 s off, 30% output. The sample was then centrifuged for 30 min at 75,600 g, 4 °C. The supernatant was loaded onto a 5 ml Bio-Rad Mini Nuvia IMAC Ni²⁺-Charged column equilibrated with equilibration buffer and washed with equilibration buffer and equilibration buffer with 20 mM imidazole until the A₂₈₀ absorbance returned to baseline. The protein was eluted using a gradient from 20 mM imidazole to 200 mM imidazole over 40 ml. Fractions were collected from all steps of the purification and aliquots from each fraction were saved for SDS-PAGE analysis.

SDS-PAGE of TIA1-LCS

An SDS-PAGE was run with aliquots from the fractions of all steps of each purification process. A BIO-RAD TGX Stain-free Fast Cast kit was used to make a 10% gel for the protein expressed in LB media. A 12% resolving, 4% stacking, gel was casted using a recipe adapted from Cold Springs Harbor Press for the protein purified from minimal media. All gel samples used 4x NuPAGE LDS loading buffer. The final samples contained 1x loading buffer containing a volume of each fraction normalized to the volume of the 20 mM wash. Samples were heated to 70 °C and then spun down at 20,000 g for 10 min and loaded into the gel. The molecular weight ladder used for both gels was the Fisher BioReagents EZ-Run *Rec* Protein Ladder.

Fibrillization of TIA1-LCS

The concentrations of protein solutions were determined using Beer's Law, a molecular weight of 15,516.97 g/mol, and an extinction coefficient of 53,860 M⁻¹cm⁻¹. The molecular weight and extinction coefficients were calculated from the protein primary sequence with the 6x His tag using the online tool ProtParam (<https://web.expasy.org/protparam/>). 200 µl of an elution fraction containing 58.86 µM TIA1-LCS purified from the LB media expression was put into a 0.5 ml Amicon Ultra

3K MWCO spin filter and centrifuged at 14,000 g for 30 min, twice, until the final volume remaining was approximately 50 μ l. The spin filter was then inverted into a new tube and spun down for 2 min at 1,000 g to collect the concentrated protein. The concentrated protein solution was mixed with 150 μ l of fibril buffer in an Eppendorf tube. The tube was rotated for 11 d. 100 μ l of the fibrillized solution was added to 300 μ l of HEPES and was sonicated using a Branson Sonifier 250 with an 1/8" tip for 5 min total using the settings 0.2 s on, 1 s off, 15% output. The fibrillized solution was sonicated to break up the fibrils and create small fibril fragments, or fibril seeds. A 65 μ M protein elution from the minimal media expression was dialyzed in 20 mM HEPES pH 7.4 buffer. 200 μ l of sample was spun down for 1 hr at 80,000 rpm and 8 °C to precipitate any aggregates. The supernatant was harvested and was combined with 75 μ l of the fibril seeds. The sample was left on the benchtop to fibrillize for 10 d.

TEM Imaging

5 μ l of the fibril solution was pipetted onto a glow discharged formvar-coated copper grid and sat for 2 min. The grid was quickly blotted with a laboratory wipe and washed twice with 5 ml of water and then negatively stained with 3% uranyl acetate for 10 s. Grids were then imaged with a JEOL 1230 electron microscope operating at 100kV with a 2k X 2k Tietz CCD camera.

Results

His-tagged TIA1-LCS can be expressed in E. coli and purified

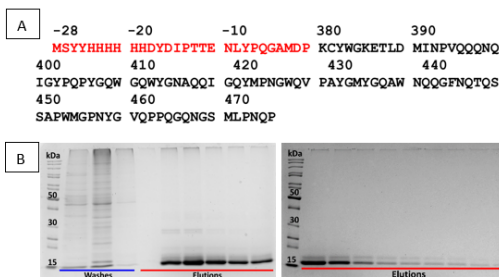


Figure 1: His-tagged TIA1-LCS preparation. (A) The single letter amino acid sequence of TIA1-LCS, with the N-terminal His-tag indicated in red. (B) SDS-PAGE of fractions from the stepwise purification procedure. The His-tagged TIA1-LCS has a calculated molecular weight of 15517 kDa.

The His-tagged TIA1 low complexity sequence domain (TIA1-LCS), with the amino acid sequence shown in Figure 1A was expressed in *E. coli* and purified using Ni^{2+} affinity chromatography with stepwise washing and elution from 20 mM to 200 mM imidazole. The purity of the TIA1-LCS protein obtained from the procedure was analyzed using the SDS-PAGE gel shown in Figure 1B. The dark bands near the bottom of the gel are consistent with the TIA1-LCS calculated molecular weight of 15517 kDa. Expression of TIA1-LCS in M9 minimal media yielded 18.22 mg from 1 l culture. Figure 2 shows that the TIA1-LCS protein obtained from the M9 minimal media is greater than 95% pure based on SDS-PAGE. Minor impurities observed in the elutions from the stepwise purification were removed using a gradient elution from 20 mM to 200 mM imidazole. Faint bands are present at approximately 16 kDa in Figure 2. Very faint bands can also be seen on some elutions in Figure 2 with a molecular weight of approximately 27 kDa.

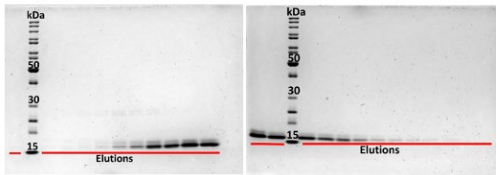


Figure 2: Preparation of TIA1-LCS in Minimal Media. SDS-PAGE of TIA1-LCS purified from M9 minimal media using a gradient elution.

Fibril Formation by His-tagged TIA1-LCS

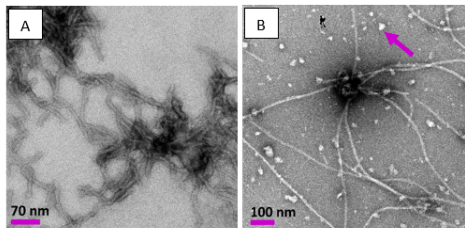


Figure 3: TEM Images of TIA1-LCS Fibrils

A. TEM images of negatively stained TIA1-LCS fibrils from initial fibril formation taken at 30,000x magnification.
 B. TEM images of negatively stained TIA1-LCS fibrils formed through repetitive seeding taken at 25,000x magnification.

Figure 3 shows micrographs of TIA1-LCS fibrils from the initial fibrillization of fibrils produced through seeded growth. The initial fibrils were bundled together and varied in length. The fibrils produced through the seeding protocol are more separated and longer than the first preparation of fibrils, but the individual fibrils from each preparation were similar in diameter and lack of a distinct twist. In Figure 3B, there appears to be amorphous aggregates, indicated with an arrow.

Discussion

In this paper we show that His-tagged TIA1-LCS can be expressed in both LB media and minimal media and purified to 95% purity using immobilized metal affinity chromatography (Figures 1B and 2). Additionally, we show that TIA1-LCS readily forms protein fibrils when at concentrations above 58 μ m in the absence of a denaturing agent or through a seeding procedure (Figure 3).

An initial concern was that the *E. coli* BL21(DE3) strain would not be able to express substantial amounts of TIA1-LCS due to toxicity of the human protein in *E. coli*. The results from the SDS-PAGE in Figure 1B confirm that we can successfully express and isolate pure TIA1-LCS using our recombinant bacterial expression system.

Once we confirmed the protein could be expressed in rich (LB) media, we investigated the expression of TIA1-LCS in minimal media (M9). Future solid state NMR measurements aimed at obtaining an atomic resolution structural model for the TIA1-LCS fibrils will require milligram quantities of isotopically labeled protein. We were able to confirm that adequate quantities of TIA1-LCS, greater than 10 mg per 1 l of culture, are obtainable in minimal media appropriate for isotopic labeling. The SDS-PAGE analysis of the protein obtained from our procedure is shown in Figure 2 and confirms that the protein is at least 95% pure.

Stepwise washing and elution of the protein was not sufficient to obtain the highly pure protein needed for structural studies. We altered our initial protocol to eliminate the impurities present in the elution from our stepwise purification protocol and found that an imidazole gradient was sufficient to remove almost all impurities. The gradient elution improved the purity of the elution, as determined by a decrease in bands on the SDS-PAGE gel that were not consistent with the molecular weight of TIA1-LCS (Figure 2).

In both gels, Figures 1B and 2, there are bands that are not consistent with His-tagged TIA1-LCS. There are faint bands right above the TIA1-LCS bands, indicating they are close in molecular weight. It is possible that it could be due to a cleavage of the His-tag. However, this cleavage would have to take place either during or after the elution step of the

purification, otherwise, the protein would not be able to bind to the column and would have been washed out before the elution. Another proposed explanation for the double band is TIA1-LCS adopting two conformations in the gel, resulting in varying amounts of interactions with SDS, altering its velocity down the gel. Part of the C-terminus of the protein may also have been cleaved during the expression of the protein, creating two different length proteins with the His-tag. The other band that is of interest is the one seen in both gels around 27 kDa. Based on the molecular weight of the monomeric TIA1-LCS, we propose that this could be a dimer of the TIA1-LCS. The formation of a dimer is unusual due to the sample containing 6 M urea and being run in SDS-PAGE, which contains a substantial amount of detergent. This proposal was made based on previous work with FUS, another protein with a LCS-domain, which runs on a gel with an apparent molecular weight of a trimer in the SDS-PAGE, despite also being solubilized in 6 M urea (Murray et al., 2017).

The TEM images, Figure 3, confirm that TIA1-LCS fibrils can be formed through seeded growth. Further optimization for fibrillization is needed to reduce the amorphous aggregates that are present in the sample. More rounds of fibrillization via seeded growth can be performed to create a more homogeneous preparation of fibrils. Amorphous aggregates could cause issues during a solid-state NMR experiment, such as inconclusive results due to signals that did not arise from the fibrils under investigation.

The data presented in this paper demonstrates that sufficient protein can be obtained for a structural investigation of TIA1-LCS. We have determined how to reliably prepare visually homogeneous fibrillar assemblies of the TIA1-LCS protein. Future work will be to record solid-state NMR measurements on the fibrils to determine the molecular conformation of the

protein in fibril form. Calorimetric, fluorescence, and denaturation assays will quantify the thermodynamics and kinetics of the fibrils. By investigating TIA1-LCS fibril assembly, we hope to understand the functional and pathogenic roles of TIA1-LCS at the molecular level.

References

- Anderson, P., & Kedersha, N. (2002). Stressful initiations. *Journal of Cell Science*, *115*(16), 3227–3234.
- Banani, S. F., Lee, H. O., Hyman, A. A., & Rosen, M. K. (2017). Biomolecular condensates: Organizers of cellular biochemistry. *Nature Reviews Molecular Cell Biology*, *18*(5), 285–298. <https://doi.org/10.1038/nrm.2017.7>
- Buchan, J. R., & Parker, R. (2009). Review Eukaryotic Stress Granules : The Ins and Outs of Translation. *Molecular Cell*, *36*(6), 932–941. <https://doi.org/10.1016/j.molcel.2009.11.020>
- Gilks, N., Kedersha, N., Ayodele, M., Shen, L., Stoeklin, G., Dember, L. M., & Anderson, P. (2004). Stress Granule Assembly Is Mediated by Prion-like Aggregation of TIA-1. *Molecular Biology of the Cell*, *15*(December), 5383–5396. <https://doi.org/10.1091/mbc.E04-08-0715>
- Kedersha, Gupta, M., Li, W., Miller, I., & Anderson, P. (1999). RNA-binding Proteins TIA-1 and TIAR Link the Phosphorylation of eIF-2 α to the Assembly of Mammalian Stress Granules. *The Journal of Cell Biology*, *147*(7), 1431–1441. <https://doi.org/10.1083/jcb.147.7.1431>
- Langenhove, T. Van, Zee, J. Van Der, & Broeckhoven, C. Van. (2012). The molecular basis of the frontotemporal lobar degeneration – amyotrophic lateral sclerosis spectrum, (February), 817–828. <https://doi.org/10.3109/07853890.2012.665471>

Lin, Y., Protter, D. S. W., Rosen, M. K., Parker, R., Lin, Y., Protter, D. S. W., ... Parker, R. (2015). Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins Article Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins. *Molecular Cell*, 60(2), 208–219. <https://doi.org/10.1016/j.molcel.2015.08.018>

Ling, S., Polymenidou, M., & Cleveland, D. W. (2013). Converging Mechanisms in ALS and FTD : Disrupted RNA and Protein Homeostasis. *Neuron*, 79(3), 416–438. <https://doi.org/10.1016/j.neuron.2013.07.033>

Mackenzie, I. R., Nicholson, A. M., Sarkar, M., Boylan, K. B., Taylor, J. P., & Rademakers, R. (2017). TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics Report TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter St, 808–816. <https://doi.org/10.1016/j.neuron.2017.07.025>

Meyer, C., Garzia, A., Mazzola, M., Gerstberger, S., Molina, H., Meyer, C., ... Tuschl, T. (2018). The TIA1 RNA-Binding Protein Family Regulates EIF2AK2-Mediated Stress Response and Cell Cycle Article The TIA1 RNA-Binding Protein Family Regulates EIF2AK2-Mediated Stress Response and Cell Cycle Progression. *Molecular Cell*, 69(4), 622-635.e6. <https://doi.org/10.1016/j.molcel.2018.01.011>

Molliex, A., Temirov, J., Lee, J., Kim, H. J., Mittag, T., Taylor, J. P., ... Kim, H. J. (2015). Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization Article Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. *Cell*, 163(1), 123–133. <https://doi.org/10.1016/j.cell.2015.09.015>

Murakami, T., Qamar, S., Lin, J. Q., Kaminski, C. F., George-hyslop, P. S., Murakami, T., ... Dong, L. (2015). ALS / FTD Mutation-Induced Phase Transition of FUS Liquid Droplets and

Reversible Hydrogels into Irreversible Hydrogels Impairs RNP Granule Function, 678–690.
<https://doi.org/10.1016/j.neuron.2015.10.030>

Murray, D. T., Kato, M., Lin, Y., Thurber, K. R., Hung, I., McKnight, S. L., & Tycko, R. (2017). Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains. *Cell*, *171*(3), 615-627.e16.
<https://doi.org/10.1016/j.cell.2017.08.048>

Renton, A. E., Chiò, A., & Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nature Neuroscience*, *17*(1), 17–23. <https://doi.org/10.1038/nn.3584>

Riback, J. A., Katanski, C. D., Kear-scott, J. L., Pilipenko, E. V., Rojek, A. E., Sosnick, T. R., ... Rojek, A. E. (2017). Stress-Triggered Phase Separation Is an Adaptive , Evolutionarily Tuned Response Article Stress-Triggered Phase Separation Is an Adaptive , Evolutionarily Tuned Response. *Cell*, *168*(6), 1028-1040.e19.
<https://doi.org/10.1016/j.cell.2017.02.027>

Ringholz, G. M., Appel, S. H., Bradshaw, M., Cooke, N. A., Mosnik, D. M., & Schulz, P. E. (2005). Prevalence and patterns of cognitive impairment in sporadic ALS. *Neurology*, *65*(4), 586 LP – 590. <https://doi.org/10.1212/01.wnl.0000172911.39167.b6>

Rowland, L. P., & Shneider, N. A. (2001). *Medical Progress 1688* . *N Engl J Med* (Vol. 344). Retrieved from www.nejm.org

Beyond the Measures Using Mobile Air Sensors to Examine Volatile Organic Compounds in Kettleman City Using a Community-Based Participatory Research Approach

Debbie Sulca¹

Mentors: Jacklyn Skye Kelty², Alfonso Aranda³ and
Dr. Clare Cannon⁴

Keywords: Disadvantaged unincorporated communities, volatile organic compounds, environmental justice, san joaquin valley, mobile air monitoring

¹Debbie Sulca, corresponding author, is a McNair Scholar and B.S. candidate in Environmental Toxicology at the University of California, Davis (email: dfsulca@ucdavis.edu; address: 1 Shields Ave., 1309 Hart Hall, Davis, CA 95616). She researches the intersections of environmental toxicology and environmental justice through multi-disciplinary perspectives.

²Dr. Jacklyn Skye Kelty received her PhD in Pharmacology and Toxicology at the University of California, Davis. ³Alfonso Aranda received his BA in History and is currently pursuing a PhD in Geography at the University of California, Davis. ⁴Dr. Clare Cannon (PI) received her PhD in Sociology from Tulane University. She researches social inequalities related to environmental justice and health using participatory research design and multiple-disciplinary approaches (email: cebcannon@ucdavis.edu; address: 1 Shields Ave., 1309 Hart Hall, Davis, CA 95616). This research is supported through pilot funding from the UC Davis Environmental Health Sciences Center (EHS CC P30ES023513). This work is also

supported by the USDA National Institute of Food and Agriculture, Hatch project #1016604 and a seed grant from the UC Davis Feminist Research Institute.

Abstract

California's Kettleman City is surrounded by industries that can emit volatile organic compounds (VOCs) into the air — agriculture, landfills, benzene treatment plants and an aqueduct. VOCs can pose severe environmental threats to residents. This research aims to examine air quality in this disadvantaged unincorporated community and understand the perceptions residents have about their environmental health using a community based participatory approach. To measure air quality, a custom built, micro-gas preconcentrator sampler was used to adsorb VOCs from indoor and outdoor environments throughout Kettleman City. Samples were analyzed via gas chromatography-mass spectrometry to putatively identify the presence of VOCs in the collected samples. Interim chemical analysis revealed potential toluene, xylene, and benzene derivatives in the outdoor air samples and potential tribromomethane in the indoor bathroom air sample. A community environmental health survey was conducted door to door over a two day period. Residents reported a negative relationship with air quality, water quality, and overall environmental health. The combination of qualitative and quantitative findings provides an overall picture of the VOCs present in Kettleman City --- adding to the story of the historical environmental racism experienced by this disadvantaged unincorporated community.

Introduction

Volatile organic compounds (VOCs) are hydrocarbon species present in the atmosphere. Although on a global scale VOC biogenic sources are more abundant than those of anthropogenic sources by approximately a factor of 10, anthropogenic sources of VOCs often dominate in urban areas (1). Major anthropogenic sources of VOCs include vehicular exhaust, fuel evaporation, industrial processes, household products, and solvent usage (2). Anthropogenic emissions of VOCs include different types of sources, such as: mobile sources, stationary sources, area sources, and point sources (3).

Atmospheric levels of VOCs are altered by various, complex, dynamic sink and source processes such as: emissions, chemical reactions, transport, and deposition (2). VOCs pose a health concern as a result of their contribution to photochemical smog and the formation of carcinogenic VOCs often generated as industrial chemicals or as byproducts of combustion. Presence and levels of VOCs were studied in Kettleman City due to residential concerns about the possible health effects these chemicals can have in their community.

This research study takes a community- based participatory research (CBPR) approach which requires researchers to acknowledge the inherent power dynamics present between researchers and community members (4). In addition, researchers collaborate with community members in choosing the research questions and throughout the research process to better understand their environmental health concerns. CBPR is thought to be a form of anticolonial advocacy scholarship that reverses the normalized roles academic research entails (5). Here, it is necessary for communities of the underrepresented and underserved peoples to voice their realities and speak for themselves as experts on daily life in

their communities. Kettleman City is recognized and referenced as an important player and forerunner in the Environmental Justice Movement (6). The California Environmental Protection Agency describes the principles of environmental justice as a call for fairness, regardless of race, color, national origin, or income, in the development of laws and regulations, implementation, and enforcement of environmental laws that affect every community's natural surroundings, and the places people live, work, play and learn (7). By working together with the community, the research team assessed how to study the environmental pollutants of interest and gather qualitative and quantitative data.

Background

Kettleman City is considered a disadvantaged unincorporated community, meeting the definition of a disproportionately low-income area that is densely settled and not within city limits (8). Kettleman City has a long history of environmental injustices and the movement towards environmental justice started in 1988 when a monumental event took place as a result of Chemical Waste Management, Inc.'s proposed installation of a toxic waste incinerator at the landfill situated near Kettleman City. This toxic waste incinerator would not only emit a myriad of VOCs but also other toxic air pollutants. With the construction of the incinerator on a landfill previously located without the residents' knowledge, the community was ready to mobilize against it (see figure 1; 6). The 1984 *Cerrell Report* was a document created for the California Waste Management Board using California taxpayer dollars to suggest locations where such companies should place their incinerators based on that location's demographics. Suggestions of the locations include communities that are rural, low-income, Catholic, had low educational levels, and were largely employed in resource extractive jobs like mining, timber, or agriculture; communities which would offer the least

resistance to such incinerators (9). For most of post-colonial American history, this unjust rationale was used to site industrial facilities and waste plants in low-resistance communities (10). Barriers that these communities may experience include: low-income, language-isolation, racism, xenophobia, and low educational attainment.

Kettleman City residents along with the help of Greenpeace fought against this implementation of an incinerator and after rigorous struggles, they successfully prevented the incinerator from being built. From this event, the community group, El Pueblo Para El Aire y Agua Limpia (“People For Clean Air and Water”) was founded, which then co-founded Greenaction for Health and Environmental Justice. Both of these community groups are still active today and worked with us throughout this research.



Figure 1. Local community members protest the Kettleman Hills Waste Facility. By Bradley Angel - Available Online, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=32066583>.

Geography and Surrounding Industries

Kettleman City is located in California’s Central Valley between Fresno and Bakersfield. The Central Valley is a flat

region surrounded by the Coast Ranges and Sierra Nevada mountain ranges, giving it a “bathtub” type of topography. This geography affects the sinks available for the emitted air pollutants and renders the Valley a closed system (6). In addition to build up of ambient VOCs based on geography, surrounding industries are also important contributors.

Kettleman City is approximately two miles off of the Interstate-5 highway (area source) and approximately three miles away from a Chemical Management Waste landfill (point source). Additionally, Kettleman City is an agricultural region bordered by agriculture on the North, East, and West side. Benzene treatment plants are also present to remove benzene from water before distribution into the public water system. All of the aforementioned emissions sources give rise to VOCs, our chemicals of interest.

Methods

Environmental Health Perceptions

To study residents’ environmental health perceptions of Kettleman City, anonymous Community Environmental Health Surveys were conducted. The research team walked to each household (N=300) with surveys on an iPad over a two day period from October 27 to October 28, 2018. Flyers with a URL link to the Qualtrics survey were left at households if no one was available to take the survey at the moment. The survey had both English and Spanish versions available. The survey had a total of 82-items regarding socioeconomic status, pesticide exposure, truck idling, female reproductive health, air quality, water quality, health concerns, and perspectives regarding law enforcement and governing agencies. Additional participant observations were recorded and documented.

Mobile Air Sensors

To study volatile organic compounds, three custom built micro-preconcentrator (μ PC) samplers were used to collect data indoors, outdoors, and within a shower. The collected data included parameters such as GPS location and time of data collection, in addition to the adhered VOCs. The air samples were collected by researchers by placing one μ PC sampler per site. Figure 2 depicts the μ PC samplers that were deployed in this pre-pilot study, indicating it was the first time the μ PC samplers were used in the field. The samples were collected on a loaded chip with a sorbent bed shown in Figure 3. The indoor data was collected within a residence and was set on a two hour timer. The indoor conditions during the time of data collection had no surrounding combustion activities at the time and were placed approximately three meters away from the kitchen area. The outdoor data was collected by placement of the sampler in a busy intersection during hot, sunny conditions ($>32^{\circ}\text{C}$) on a two hour timer. The shower sample was collected by placing the sampler in a shower setting with cold running water for 30 minutes. The air samples were then analyzed using gas chromatography-mass spectrometry (GC/MS). The GC/MS used was a Varion ion trap 3800GC/4000MS.

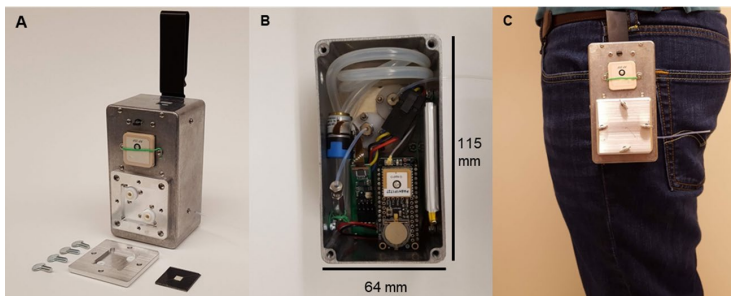


Figure 2 Custom built μ PC sampler, which collects VOCs from air. A) Thumbscrews allow users to load chips for multiple VOC collections B) The sampler contains a pump, microcontroller and GPS for sample collection C) The device is mobile and even wearable (11).

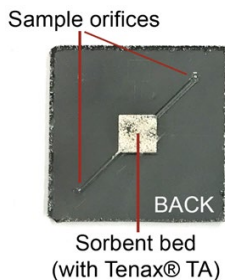


Figure 3 The μ PC sampler (above) passes air samples through these custom chips, which are packed with a chemical sorbent. The sorbent adsorbs VOCs from the air and is returned to the lab for chemical analysis via GC-MS (12)

Results

The Household Community Environmental Health Survey received an 11% response rate (N=31).

Demographics

Of the people that responded, 64% identified as female and the remaining 36% identified as male. 57% of participants have resided in Kettleman City for over 12 years, 14% have lived in Kettleman City for 6-12 years, 21% for less than six years, and 7% for less than a year. 55% of participants either work or live with a household member that works in agriculture. Out of those that responded to the survey question (n=28), 35% had no children under the age of 18 years residing there, 18% had one, 25% had two, 21% had three minors or more. Out of the 31 contributors, roughly 55% chose to take the survey in English. However, 74% of participants' households primarily speak Spanish in their household while the remaining 26% primarily speak English.

Environmental Health Concerns

When asked about the level of environmental pollution in their neighborhood, 84% of those who responded (N=31) answered

somewhat high, high, or very high while 16% responded somewhat low or low. Participants were then asked to identify the most concerning environmental health problems in their community using a list of 13 choices. Of those who responded (n=22), the most frequently reported problems included asthma (19%), valley fever (12%), birth defects (11%), respiratory illnesses (11%), and cancers (11%). Of least concern was neurological disorders (3%), development disorders (1%), and injuries or accidents (1%). For the following question (n=27), 55% of participants reported having observed a truck idling in their community, 19% answered that they had not seen a truck idling in their community, and 13% were unsure. When asked if there are time limits to truck idling, 10% responded yes, 57% responded no, and 30% responded that they did not know.

Gas Chromatography- Mass Spectrometry

The GC/MS chromatogram depicted in Figure 4, together with the instrument's library, showed that toluene, xylene, and benzoyl chloride were possibly present in the outdoor sample. The indoor sample contained possible toluene and benzoyl chloride. Tribromomethane was possibly detected in the shower sample. Caveat: This pre-pilot data collection and small sample size provide putative identifications only.

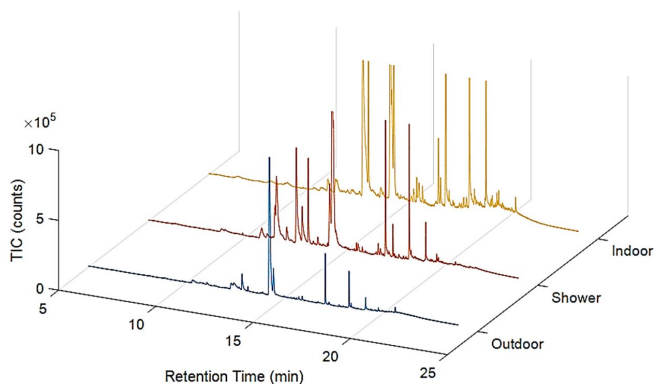


Figure 4 GC/MS chromatogram TIC (counts) vs Retention Time (min)-- unpublished result. Comparison of data collected from three locations within Kettleman City.

Discussion

The Community Environmental Health Survey was used to get a baseline understanding of the emission sources of VOCs and residential perceptions of environmental health. Out of the provided options of environmental health concerns, air quality-related ailments were among the most concerning for residents. Although possible identifications of the VOCs present in Kettleman City were provided by the GC/MS data, we cannot state whether these airborne chemicals are at toxic levels without quantification of VOC concentrations-- a critical step in predicting their environmental and health impacts. However, based on the putative identifications of the air samples, the GC/MS identified possible benzoyl chloride present in both outdoor and indoor environmental samples. No atmospheric reactions are known to produce benzoyl chloride. Thus, if benzoyl chloride were present both in indoor and outdoor environments, it would be due to industrial emissions. Once released to the atmosphere, benzoyl chloride can react with hydroxyl radicals or photolyze. However, benzoyl chloride exists as a fuming liquid and can volatilize from dry soil. Benzoyl chloride's shortest lifetime has been determined to happen via hydrolysis, thus making rain a possible major sink for the possible omnipresent compound in Kettleman City (13).

Toluene was also present in both outdoor and indoor environments. Toluene is a component of gasoline among other solvents and is found in cigarette smoke. Together with xylene and benzene (BTX), the mixture is used as an additive for gasoline (14). Once in the atmosphere, toluene can be degraded photochemically by hydroxyl radicals. The variability of sources for this chemical reflects the possible presence of it in both outdoor and indoor environments.

Xylene was found to be possibly present in the outdoor sample and not the indoor sample. Xylenes include various isomers and can be emitted to the air with automobile exhaust, paints, coatings, and adhesives (15). Xylene is also a combustion byproduct emitted from gas engines, diesel engines and burning of wood. Once in the atmosphere, xylene can react with hydroxyl radicals or be removed from the air via wet deposition (16). Since xylene was identified only in the outdoor sample, likely sources in Kettleman City are environmental from fuel combustion and exhaust.

Lastly, tribromomethane or bromoform is a byproduct of water disinfection (17). The cold shower sample provided a peak for bromoform in the chromatogram picture in Figure 4. Cold water was selected primarily to reduce the cost for the resident and also to reduce the amount of volatiles present during the sample collection. Tribromomethane is mostly degraded in the atmosphere by hydroxyl radicals and is not susceptible to photolysis (17).

Reflecting on the survey responses, residents reported encountering the emission of air pollutants through the survey responses. An emission source to highlight is truck idling, which can be responsible for the putative findings of xylene and toluene. Additionally, VOC concentrations indoors are affected by ventilation. Many residents expressed keeping windows and doors closed for most of the day in order to keep out harmful volatile compounds and few participants mentioned having an air filter within the home. These observations indicate a possibility of higher than usual VOC concentrations within households. The benefit of using the μ PC samplers was their small size. This method was not only appropriate since it included a Tenax® sorbent bed for the collection of VOCs but its mobility made the collection process simple (18).

A limitation for this study was low participants for surveys. In part, this was due to the mistrust that some residents have towards researchers from an institution. An additional limitation was the distance between researchers and the community leading to a partnership with few face-to-face interactions. However, a benefit from using CBPR were the interactions between researchers and community members that were eager to participate. The community members that were interested in the study provided guidance and knowledge to the researchers during the study. The use of the CBPR method was of great importance to this interdisciplinary study and allowed for the research to be problem-based instead of discipline driven, a comprehensive approach to complex problems (19). Together, qualitative and quantitative results revealed the presence of VOC emissions in the community as identified by residents and gas chromatography. Not surprisingly, residents hold negative perceptions of their environmental health in Kettleman City. More representative VOC data collection is necessary to more confidently identify and quantify the VOCs present and exposed to residents in this environmentally disadvantaged community in California's Central Valley.

Conclusion

When discussing environmental pollution and the effects of air quality, the demographics of the locations with these emissions sources and the experiences of fence-line communities near these sources are oftentimes disregarded. This lack of awareness regarding the experiences that people face as a result of environmental injustices creates a knowledge gap in the general populace and slows down the process for creating policies to combat these point-source contributors to environmental pollution at the core. By including CBPR methods into scientific studies, scientists humanize the data and are better able to effectively communicate the urgency to either

finding alternatives to existing practices or discontinuing them entirely. By intertwining environmental justice with environmental science, a myriad of solutions may arise. Considering that this research focuses on environmental health, future research should include studying ground-level ozone levels, quantification of nitrous oxides, analysis of the air filters some residents have within the residence, water quality, and biological studies of levels of toxicity. Future studies for this project on air quality include, quantification of VOCs, as well as pesticide drift and particulate matter. In addition, our study will include interviews with Kettleman City residents that will be recorded and transcribed.

Acknowledgements

This project was made possible due to collaborations with community partners, scientist collaborations, and funding. The community partners from El Pueblo Para El Aire y Agua Limpio and Greenaction for Health and Environmental Justice, a special thank you to Maricela and Miguel. The micro-preconcentrator samplers were designed built at Dr. Cristina Davis' lab, UC Davis Bioinstrumentation and BioMEMS Laboratory. The VOC data was also analyzed at Davis' lab using their GC/MS. A special thank you to Mitch McCartney, Maneeshin (Yasas) Rajapakse, Alexander (Zander) Fung, Fauna Fabia, and Leslie Simms for their mentorship in the lab. This research is supported through pilot funding from the UC Davis Environmental Health Sciences Center (EHS CC P30ES023513).

References

1. Atkinson, R. & Arey, J. (2003). Atmospheric Degradation of Volatile Organic Compounds. *Chemical Reviews*, 103 (12), 4605-4638. DOI: 10.1021/cr0206420

2. Guo, H., Ling, Z.H., Cheng, H.R., Simpson, I.J., Lyu, X.P., Wang, X.M., Shao, M., Lu, H.X., Ayoko, G., Zhang, Y.L., Saunders, S.M., Lam, S.H.M., Wang, J.L., and Blake, D.R. (2017). "Tropospheric Volatile Organic Compounds in China." *Science of the Total Environment* 574, 1021-043. Web.
3. Borbon, A., Gilman, J. B., Kuster, W. C., Grand, N., Chevallier, S., Colomb, A., ... & Holloway, J. (2013). Emission ratios of anthropogenic volatile organic compounds in northern mid-latitude megacities: Observations versus emission inventories in Los Angeles and Paris. *Journal of Geophysical Research: Atmospheres*, 118(4), 2041-2057.
4. Israel, B. A., Schulz, A. J., Parker, E. A., & Becker, A. B. (2008). Critical Issues in Developing and Following Community-Based Participatory Research Principles. In M. Minkler, & N. Wallerstein (Eds.), *Community-Based Participatory Research for Health* (pp. 47-62). San Francisco, CA: Jossey-Bass.
5. Deeb-Sossa, N. & Rodriguez, L. (2019). Community-based participatory research: testimonios from Chicana/o studies, *The University of Arizona Press*, 5-7.
6. Cole, L. W., & Foster, S. R. (2001). *From the ground up: Environmental racism and the rise of the environmental justice movement* (Vol. 34). NYU Press.
7. Peter, E. M. (2001). Implementing Environmental Justice: The New Agenda for California State Agencies. *Golden Gate UL Rev.*, 31, 529.
8. Flegal, C., Rice, S., Mann, J., & Tran, J. (2013). California Unincorporated: Mapping Disadvantaged Communities in the San Joaquin Valley. Retrieved from

https://www.policylink.org/sites/default/files/CA_UNINCORPORATED_FINAL.pdf.

9. Cole, L. W. (1994). The Struggle of Kettleman City: Lessons for the Movement, *Maryland Journal of Contemporary Legal Issues* 5(1), 67-80.
10. Bullard, R. D. (1993). Environmental racism and invisible communities. *W. Va. L. Rev.*, 96, 1037.
11. Fung, A. G., Rajapakse, M. Y., McCartney, M. M., Falcon, A. K., Fabia, F. M., Kenyon, N. J., & Davis, C. E. (2019). Wearable Environmental Monitor To Quantify Personal Ambient Volatile Organic Compound Exposures. *ACS sensors*.
12. McCartney, M. M., Zrodnikov, Y., Fung, A. G., LeVasseur, M. K., Pedersen, J. M., Zamuruyev, K. O., ... & Davis, C. E. (2017). An easy to manufacture micro gas preconcentrator for chemical sensing applications. *ACS sensors*, 2(8), 1167-1174.
13. Hazardous Substances Data Bank [Internet]. Bethesda (MD): National Library of Medicine (US); 1989- [updated 2018, Dec 18; cited 2019] Benzoyl chloride; Hazardous Substances Databank Number: 98-88-4. Available from <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+383>
14. Hazardous Substances Data Bank [Internet]. Bethesda (MD): National Library of Medicine (US); 1985- [updated 2016, Oct 25; cited 2019] Toluene; Hazardous Substances Databank Number: 108-88-3. Available from <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~tDX4Oo:1>

15. Grosjean, D. (1991). Atmospheric fate of toxic aromatic compounds. *Science of the total environment*, 100, 367-414.
16. Hazardous Substances Data Bank [Internet]. Bethesda (MD): National Library of Medicine (US); 1986- [updated 2016, Oct 25; cited 2019] Xylenes; Hazardous Substances Databank Number: 1330-20-7. Available from <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~LSbRnz:1>
17. Hazardous Substances Data Bank [Internet]. Bethesda (MD): National Library of Medicine (US); 1984- [updated 2019, May 31; cited 2019] Bromoform; Hazardous Substances Databank Number: 75-25-2. Available from <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~Unj0tm:1>
18. European Commission (1995). Sampling strategies for volatile organic compounds (VOCs) in indoor air.
19. Bacon, C., deVuono-Powell, S., Frampton, M. L., LoPresti, T., & Pannu, C. (2013). Introduction to empowered partnerships: Community-based participatory action research for environmental justice. *Environmental Justice*, 6(1), 1-8.

How Genome-Wide Association Studies on Educational Attainment Reify Eugenic Ideologies

Dawn Warfield*

Mentor: Dr. Emily Merchant
Science and Technology Studies, UC Davis

Abstract

Genomic science and data are becoming ubiquitously entwined with how we describe the human condition in its physical, psychological, and social expression. Behavioral heredity research delves into genetic data science to reveal possible conditions, correlates, and determinants for particular behaviors and aptitudes that shape our individual and collective human experience. This article surveys the history, research culture, and methods of behavioral heredity science to investigate the techno-social interaction and motivations for recent genome-wide association studies (GWAS) performed to identify genomic biomarkers associated with educational attainment.

Introduction

The knowledge regime of behavioral heredity began in the late 19th century with Mendelian inference and extrapolated findings from animal breeding. As the technology of molecular biology enabled new understandings about the relationship between the heritability of traits and medical conditions through genes, social scientists began to explore genetics as a means to biologically explain human behavior and socioeconomic potential. In the most recent iteration of

behavioral heredity, sociogenomic researchers now have access to both genomic and empirical survey data derived from longitudinal studies with broadening population samples, engendering new possibilities for finding associations.

A Historical Perspective on Sociogenomics

The longer history of sociogenomics begins with Francis Galton, who coined the word “eugenics,” literally meaning “well borne” in Greek, in 1883 (Müller-Wille and Rheinberger). Galton’s belief that human society could be perfected through selective breeding and social divestment of those considered to be a threat to the gene pool proved popular among scientists at the turn of the twentieth century. Eugenics had a synergistic relationship with the era’s scientific racism, epitomized in Harvard anthropologist Louis Agassiz, who insisted that black brains had lower capacities and that educating them could cause brain damage or even death (Beckwith). Eugenics gained momentum to shape US immigration policy. Hereditarian studies purporting that immigration would pollute white progeny with deleterious results like lower birth rates, worse infant health, and impaired intelligence held sway in enacting public policies that restricted immigration from non-northern European countries (Ngai). From 1907 until 1979, over 30 states passed eugenic sterilization laws and about 60,000 procedures were done (Stern). The 1927 Supreme Court case *Buck v. Bell* added legitimacy to eugenics. The forced sterilization of poor, black, and immigrant women was often rationalized by “feeblemindedness,” a slippery category that claimed a basis in heredity (Stern). The popularity of eugenics waned after WWII, when it became indelibly associated with Nazism, but it didn’t exactly disappear. To recover the legitimacy of the eugenic project, its American supporters turned their attention to medical genetics.

Beginning in the middle of the twentieth century, molecular biology engendered new technologies for empirical study of the gene. These empirical tools, however, did not make their way into behavior genetics, which emerged in the 1960s to quantify the “heritability” of given social traits and outcomes through phenotype analysis in twins (Panofsky). As American society underwent great changes in the late 1960s and early 1970s, marked by second wave feminism and the civil rights movement, supporters of the status quo cited research in behavior genetics to assert that inequality of socio-economic outcomes was rooted in biological differences in ability. In 1969, educational psychologist Arthur Jensen extrapolated from his research to claim that programs like Head Start would never overcome IQ differences between white and black children because the majority of the difference was genetic (Jensen). In 1994, the controversial book “The Bell Curve,” written by political scientist Charles Murray and psychologist Richard Herrnstein, purported that there is great disparity between the races in their biologically determined abilities. Murray and Herrnstein utilized biological determinism to provide rationale for fiscal conservatives to argue for the divestment of social welfare programs that they claim encourage low IQ women to breed and be supported by society (Herrnstein and Murray). Behavior heredity also sought evidence of genetic determinism to explain gender inequalities. In 1979 educational psychologists Camilla Benbow and Julian Stanley hypothesized “superior male mathematical ability, which may in turn be related to greater male ability in spatial tasks” (Benbow and Stanley). This claim received considerable media coverage (Williams and King). Supporters saw in Benbow and Stanley’s work a “math gene” that men had and women did not, while critics pointed to differences in exposure to math between girls and boys (Beckwith).

In the 1990s, the Human Genome Project (HGP) began with the aim to fully sequence the human genome. Supporters expected that the project would produce the key to all things human, but instead it revealed further complexity with huge spectrums of possibilities for genomic contributions and environmental adaptations (Reardon). The epic scale of the project catalyzed an inter-institutional cooperative endeavor, with numerous public and private investors investing millions of dollars into equipment and analysis, but researchers grappled with how to generate a return on investment from the mass of information the HGP produced (Reardon). The HGP made the aspirational and inclusive discovery that humans are all more than 99% the same, but researchers immediately turned to the differences among us, spawning new projects like the Human Genome Diversity Project (HGDP), to probe those differences (Reardon). What the HGP and HGDP did effectively was drive researchers to gather big data on human genetic variation and forge techniques for making knowledge of the genome more functionally predictive. Genomic data, and not hereditary inference, would prove to be a great advancement in understanding human variation, and subsequently utilized by behavior heredity science to biologically explain how genes contribute to human performance.

GWAS and PGS

Since the completion of the HGP, the promise of genomic data has compelled social scientists to collect genetic samples along with surveys of population outcomes, enabling the current era of data-driven sociogenomics and genome-wide association studies (GWAS). GWAS originated in medical studies, where the technique has succeeded in identifying regions of the genome with genes that have causal effects on health. This research strategy focuses on the less than 1% of the human genome that is known to differ from person to person—single nucleotide polymorphisms—or SNPs for short. GWAS

correlate SNPs from across the genome to phenotypes of interest in large sample populations. This strategy has been very successful in expanding our understanding of diabetes and identifying the BRCA gene associated with breast cancer (Billings and Florez; Kraft and Haiman).

Social scientists use GWAS to correlate SNPs with social traits or outcomes. The strength of GWAS SNP correlations is highly dependent on having huge genetic samples with information on measurable traits that are of interest to the researcher. Because GWAS requires samples of 100,000 or more, they are typically done by consortia, which meta-analyze findings from separate studies spanning the social, medical, and commercial realms. The studies of educational attainment that are the focus of this article were all done by the Social Science Genetic Association Consortium (SSGAC), which was founded in 2011 by a group of economists. In addition to promoting research, the SSGAC works to build the field of sociogenomics through workshops for postdocs and young faculty members at the Russell Sage Foundation's Summer Institute for Social-Science Genomics. Between 2013 and 2018, The SSGAC produced three GWAS to identify SNP correlations for educational attainment. These studies focused on educational attainment, as this was a phenotype that was consistently available across all samples, but they treat educational attainment as a proxy for socioeconomic success, which was the original target of eugenics. As these studies grew in size from just over 100,000 participants in 2013 to over 1.1 million in 2018, the number of SNPs found to be statistically associated with educational attainment expanded from 3 to 1,271. The SSGAC used the findings from these GWAS to construct polygenic scores (PGS), which quantify an individual's genetic association with a measurable phenotype expression, in this case educational attainment. The headlines were flashy, announcing that more than a thousand genetic loci are related to educational

attainment (Rayner et al.), but the fine print was less exciting. The SNPs with the strongest association corresponded to a difference of about three weeks of additional education (Alonso). Summed into a PGS, these findings could explain only about 12% of the variance in a sample of European Americans, and very little of the variance among nonwhites (Okbay et al.). Subsequent research found that parental income was a better predictor of educational attainment than the PGS for educational attainment (Martin). On an individual level, the PGS is not useful for predicting the educational attainment of any given person (Zimmer).

Like earlier iterations of behavioral heredity science, the inclusion of nonwhites in the process of establishing normative genomic metrics – or in this case, their non-inclusion – continues to be problematic. Informed by the field of population genetics, GWAS are typically done on samples that are homogeneous in terms of genetic ancestry. All of the educational attainment GWAS were limited to people of European descent, and efforts to apply their findings to nonwhites have largely failed. The GWAS catalogue reports that 79% of all GWAS participants are of self-reported European descent, even though they make up only 16% of the global population (Martin et al.). Further complicating the issue, geneticists have found that studies on Hispanic/Latino and African American individuals show that these populations “contribute an outsized number of associations relative to studies of similar sizes in Europeans” (Martin et al.). Inclusive studies would have to be done on large samples of people of color in order to generate comparable results, and large-enough samples currently don’t exist (“Genetics for All”). Therefore, whatever these studies identify as optimal genomic metrics for educational attainment are modeled by and only relevant to homogenous white populations.

Social Implications

Scientific findings don't need to be substantiated to take hold in society. Let us reconsider Benbow and Stanley's "math gene." Media coverage of this spurious finding affected parental perceptions and expectations about the mathematical abilities of their children. A subsequent study by Jaqueline Eccles and Janis Jacobs in 1995 on the effect of exposure to the math gene media found that fathers exposed to misinformation felt confirmed that math wasn't as important for their girls as it was their boys and continued and/or lowered their expectations for their daughters' math ability. Mothers absorbed the information personally, believing they weren't as good at math and/or as intelligent as men and projected these beliefs onto their daughters (Eccles and Jacobs). Research in social psychology has shown that women will do less well on a math test if they are reminded of their 'womanness' before taking the test, simply by being asked to mark 'M' for male or 'F' for female on the test, or by being in a room full of men, whereas men will do better on the test if they are reminded of their "maleness" (Fine).

In review of the history of behavioral heredity science, a few patterns emerge. Claims of behavioral heredity are often used by political agents who wish to reinforce negative stereotypes of non-whites, women, and the poor in order to support policy arguments for divestment from social welfare programs that are implemented as safety nets and to mediate equity of opportunity. Genomic studies surface new claims of determinative genetics and the media embeds them into the communal social ethos. Once an idea is popularized, it's very difficult to get rid of. For example, the Graduate Records Exams (GRE) are known to have very little power to assess student intelligence or predict how well you will do in graduate school and the College Board isn't even a formal educational or governmental institution—but there continues to be a growing

industry that's literally banking on the fears of students that their GRE score determine their future success.

The inference that genomic associations can determine socioeconomic outcomes fuels the development of new products for people seeking to explain their behavior through science, gain a competitive edge, or even select mates that will produce desired traits in their progeny (Conley and Fletcher). At-home DNA test companies share your data with genomic consortiums to advance the field of genomic prediction, which helps to create new curious customers and products. There are new products in development seeking to capitalize on the idea of genetic prediction, like EduCred, which is positioning itself to apply the model of utilizing genetic data for precision medicine to create products and methods for "precision academics" (EduCred). There are institutional, financial, and political stakeholders seeking gains through genomic data, making the temptation of the low-hanging fruit of philosophical fallacy a real peril.

Conclusion

Philosopher John Dewey warned that "the most pervasive fallacy of philosophical thinking goes back to neglect of context" (Dewey). In light of our current political climate and finding ourselves on the precipice of CRISPR-enabled genomic interventions, George Pappas's analysis of John Dewey's ethics illuminates four aptly important philosophical fallacies (Pappas). The first, "The Analytic Fallacy," is where results are interpreted as complete within themselves (Pappas). Polygenic scores should not stand alone to predictively determine an individual's abilities or potential. The second, "Unlimited Universalization," is when context is ignored and conclusions are elevated to imply universal application (Pappas). Polygenic scores do not include people of color and are thus far too

limited in their scope and specificity to be meaningfully applied to entire populations. Media that reports findings as if they apply to all with little attention to the fine print can mislead the public. Pappas's third fallacy, "Selective Emphasis," happens when the selectivity and purpose of selection is ignored in the context of an inquiry (Pappas). The limitations of the SSGAC's samples and methodological strategies, as well as their research motivations, are not wholly transparent in their articles. Their studies and training activities are funded by organizations including the Russell Sage Foundation and the Ragnar Söderberg Foundation, which provide capital to support their social policy aims. This information provides important contextualization for their scientific deliverables. The last is "The Intellectualism Fallacy," a combination of the previous three fallacies, where the pre-cognitive and non-cognitive are dismissed (Pappas). Dewey advises us to remember that "the world in which we immediately live, that in which we strive, succeed, and are defeated is preeminently a qualitative world" (Dewey). When researchers attempt to quantify that which is qualitative, like home environment and educational differences, and merely plug in numbers to represent abbreviated personal histories, they diminish the role of environment and exaggerate the role of biological determinism.

Context is extremely important when gauging whether the risks are worth the gains when undertaking studies and disseminating claims. Given that PGS on educational attainment are only derived from populations with European origins, and that the scholars who perform GWAS are not following up by investigating how particular genomic loci are associated with learning outcomes, it becomes quite credible to question the purposes and benefits of GWAS on educational attainment. The PGS does not provide medical or social information towards ameliorating educational deficits. From a practical standpoint, it would follow that institutions interested

in improving learning outcomes could support social welfare and education programs with the resources and capital spent on crafting normative polygenic scores that have little individual predictive value. Behavior heredity science has fallen prey to all of the above pitfalls throughout its history, and sociogenomics must be conscientious and clearly acknowledge and communicate the limitations and motivations of genomic data analysis performed to derive predictors of socio-economic outcomes.

The future of sociogenomics is rife with potential medical, social, and political implications (Bliss, *Social by Nature*). Genetic technology is currently ahead of ethical policy development. Sociogenomics may contribute to racialization and genetic stereotyping. Polygenic scores could become a new metric for societal marginalization and increase stress on currently marginalized sectors if they are used to provide rationale for allocating a greater share of resources to people who are already advantaged. Sociogenomics has the potential to impact the personal identity and psychological well-being of people diagnosed with genetically determined traits. Being labeled with scores may inculcate ideas about genetic superiority and inferiority and cause people to think they are either better than others, or that they can't learn or achieve beyond the genomic potentials ascribed to them (Bliss, *Race Decoded*). There is clearly a need for further research on genome-wide association studies, including: 1) the history of the field's cultural and methodological development through eugenics and behavior genetics and assumptions that may be embedded within methods for constructing polygenic scores that predict educational attainment and economic success; 2) the effects of transmission and re-situation of behavioral genomic correlations from research settings into knowledge and products for the public, and; 3) the personal and socio-

political benefits and implications of polygenic scores that claim to predict an individual or group's educational potential.

References

Alonso, Jose R. "Genetics of Educational Attainment." *Mapping Ignorance*, <https://mappingignorance.org/2018/09/26/genetics-of-educational-attainment/>. Accessed 24 Nov. 2019.

Beckwith, Jon. "Gender and Math Performance: Does Biology Have Implications for Educational Policy?" *The Journal of Education*, vol. 165, no. 2, 1983, pp. 158–74, doi:10.2307/42772831.

Benbow, C., and J. Stanley. "Sex Differences in Mathematical Ability: Fact or Artifact?" *Science*, vol. 210, no. 4475, Dec. 1980, pp. 1262–64, doi:10.1126/science.7434028.

Billings, Liana K., and Jose C. Florez. "The Genetics of Type 2 Diabetes: What Have We Learned from GWAS?" *Annals of the New York Academy of Sciences*, vol. 1212, no. 1, Nov. 2010, pp. 59–77, doi:10.1111/j.1749-6632.2010.05838.x.

Bliss, Catherine. *Race Decoded : The Genomic Fight for Social Justice - 01UCD*. Stanford University Press, 2012, https://search.library.ucdavis.edu/primo-explore/fulldisplay?docid=01UCD_ALMA21192436640003126&context=L&vid=01UCD_V1&search_scope=everything_scope&isFrbr=true&tab=default_tab&lang=en_US.
---. *Social by Nature: The Promise and Peril of Sociogenomics*. 2018.

Conley, Dalton, and Jason Fletcher. *The Genome Factor : What the Social Genomics Revolution Reveals about Ourselves, Our History, and the Future - 01UCD*. Princeton University Press, 2017, https://search.library.ucdavis.edu/primo-explore/fulldisplay?docid=01UCD_ALMA21283994450003126&context=L&vid=01UCD_V1&search_scope=everything_scope&tab=default_tab&lang=en_US.

Dewey, John. *The Later Works, 1925-1953*. Edited by Jo Ann Boydston, Carbondale : Southern Illinois University Press, 1981.

EduCred. *What Precisely IS Precision Academics? — EduCred Services*. <http://educredservices.com/blog/2017/4/27/what-precisely-is-precision-academics>. Accessed 10 Nov. 2019.

Fine, Cordelia. *Delusions of Gender : How Our Minds, Society, and Neurosexism Create Difference*. 1st ed., New York : W. W. Norton, 2010.

“Genetics for All.” *Nature Genetics*, vol. 51, no. 4, 2019, p. 579, doi:10.1038/s41588-019-0394-y.

Jensen, Arthur. “How Much Can We Boost IQ and Scholastic Achievement?” *Harvard Educational Review*, edited by Arthur Jensen, vol. 39, no. 1, 1969, pp. 1–123, doi:10.17763/haer.39.1.13u15956627424k7.

Kraft, Peter, and Christopher A. Haiman. “GWAS Identifies a Common Breast Cancer Risk Allele among BRCA1 Carriers.” *Nature Genetics*, vol. 42, no. 10, Nature Publishing Group, 2010, pp. 819–20, doi:10.1038/ng1010-819.

Martin, Alicia R., et al. “Clinical Use of Current Polygenic Risk Scores May Exacerbate Health Disparities.” *Nature Genetics*, vol. 51, no. 4, Nature Publishing Group, Apr. 2019, pp. 584–91, doi:10.1038/s41588-019-0379-x.

Martin, Nicholas. “Getting to the Genetic and Environmental Roots of Educational Inequality.” *Npj Science of Learning*, vol. 3, no. 1, Nature Publishing Group, Dec. 2018, p. 4, doi:10.1038/s41539-018-0021-1.

Müller-Wille, Staffan, and Hans-Jörg. Rheinberger. *A Cultural History of Heredity*.

Ngai, Mae M. “The Architecture of Race in American Immigration Law: A Reexamination of the Immigration Act of 1924.” *The*

Journal of American History, vol. 86, no. 1, 1999, pp. 67–92,
doi:10.2307/2567407.

Okbay, Aysu, et al. “Genome-Wide Association Study Identifies 74 Loci Associated with Educational Attainment.” *Nature*, vol. 533, no. 7604, Nature Publishing Group, May 2016, pp. 539–42,
doi:10.1038/nature17671.

Pappas, Gregory Fernando. *John Dewey’s Ethics : Democracy as Experience*. Bloomington : Indiana University Press, 2008.
Rayner, Christopher, et al. “Genetic Influences on Treatment-Seeking for Common Mental Health Problems in the UK Biobank.” *Behaviour Research and Therapy*, Pergamon, June 2019, p. 103413,
doi:10.1016/J.BRAT.2019.103413.

Reardon, Jenny. *The Postgenomic Condition*. University of Chicago Press, 2017, doi:10.7208/chicago/9780226345192.001.0001.

Stern, Alexandra Minna. “That Time The United States Sterilized 60,000 Of Its Citizens | HuffPost.” *Zocolo Public Square*, 2016.

Williams, Dennis A., and Patricia King. “Do Males Have a Math Gene?” *Newsweek*, 1980, p. 73,
[http://beck2.med.harvard.edu/week6/Newsweek Time.pdf](http://beck2.med.harvard.edu/week6/Newsweek%20Time.pdf).

Zimmer, Carl. “Years of Education Influenced by Genetic Makeup, Enormous Study Finds.” *The New York Times*, 23 July 2018,
<https://www.nytimes.com/2018/07/23/science/genes-education.html>.

Locked Down: The Hidden History of the Prisoners' Rights Movement

Tiana Alexandria Williams*

Mentor: Dr. Jesse Drew
Department of Cinema and Digital Media, UC Davis

Abstract

In recent years, there has been an increasing discourse centered on the prison-industrial complex, addressing issues that range from ending the school-to-prison pipeline to calls for the abolition of prisons entirely. However, this movement is far from a novelty, rather, it is the resurgence of a forgotten moment in history that is being revitalized by a new generation. In order to understand the recent development of the anti-incarceration movement, it is important to provide context to these current conversations and ensure that the contributions of the prisoner's rights movement are properly understood. Through the uncovering and analysis of archival materials, collections of recorded oral histories and published prison letters, this paper illustrates how prisoner activism of the 1970s brought the plight of prisoners into the limelight, while also leading to increased systemic repression and a debilitating historical declension narrative. By highlighting this history of prisoner activism, this paper challenges the declension hypothesis approach to the prisoners' rights movement and investigates the movement's effects on the current day structure of the criminal justice system.

Introduction

The prisoners' rights movement is an undermentioned and understudied movement that grew for decades in the United States, finally reaching maximum visibility in the early 1970s. The dominant narrative surrounding the movement can be traced as: *The American prison system was cruel, inhumane and unjust. Prisoners fought for better conditions and rights, using similar rhetoric to that of the Civil Rights Movement. The movement declined simultaneously with the courts and states' responses to legal pushes for rights, and many of the more humane institutional norms seen today are a product of prisoner activism of the 60s and 70s.* Heather Ann Thompson, author of *Blood in the Water: The Attica Prison Uprising of 1971 and Its Legacy* speaks to the issues of the narrative surrounding the prisoners' rights movement stating that "in key instances, the prisoner activism of the 1960s and 1970s brought fundamental improvements to institutions of punishment around the country, and in other respects they indeed fueled a hostility that served to net them even more unconscionable abuse. To write the history of this period fully, scholars must wade into this complexity."¹ This charge from Heather Ann Thompson has influenced my investigation into the declension narrative surrounding the prisoners' rights movement and its relationship to systemic repression within prisons today. For this paper, a declension narrative is being defined as any story of change overtime that traces a secular decline or deterioration in historical phenomenon, or any story that is told in a non-cyclical way. When forming my initial research question, I heavily considered the onset of mass incarceration, presuming that the phenomenon was a byproduct of the prisoners' rights

¹ This statement from Heather Ann. Thompson is taken from *Process*, the blog of the Organization of American Historians, *The Journal of American History*, and *The American Historian*. The quote is from a roundtable discussion with scholars of the carceral state about prisoners organizing in the 1960s and 1970s.

movement and backlash for this activism. However, Robert T. Chase, an assistant professor of history at Stony Brook University, mentions in his paper *We Are Not Slaves: Rethinking the Rise of Carceral States through the Lens of the Prisoners' Rights Movement* that mass incarceration was not necessarily a product of prisoner activism of the 1960s and 1970s, but instead prisoners saw this phenomenon emerging and sought to curtail it. Chase furthers this idea by explaining how “a full accounting of the rise of the carceral state must look at how prisoners sought to counter the rising tide of mass incarceration [and that] continuing this research and excavating multiple histories of prisoner resistance might well offer a path to confront the ways a variety of carceral states have taken such deep root across American politics and society.”² This research seeks to look deeper into this complexity that Robert T. Chase describes and through reviving and analyzing archival material containing primary sources, collections of recorded oral histories, and reviewal of secondary sources this paper will examine how the declension narrative surrounding the movement has been *created* and *maintained* through state tactics such as censorship, discreditation of prison activists, and a concealment of political prisoners in the United States.

The purpose of this project can be simplified to these three terms: to examine, document and combat. The overall project seeks to examine the prisoners' rights movement in California and its impact on the present-day prison system, which includes investigating the ways in which the movement fueled a hostility that produced systemic backlash and repression as well as a historical declension narrative. Moreover, the project works to document oral histories and revive the hidden archive and focus on identifying and preserving these histories that have been obscured. Lastly, but most importantly, this research intends to

² Chase, Robert T., “We Are Not Slaves: Rethinking the Rise of Carceral States through the Lens of the Prisoners' Rights Movement.” (2015) 73–86.

combat the declension narrative surrounding the movement. In short, this project seeks to bring this little-known history of the prisoners' movement to the forefront of present-day conversations regarding the prison system in order to think deeper about the ways it has contributed to forming the present-day prison and criminal justice system.

Case Studies: George Jackson, the Soledad Brothers and the San Quentin Six

When I first began this research project, I had never heard about the prisoners' rights movement that shook the country throughout the 1960s and the decades following. This realization sparked a great interest in researching the background and context surrounding the movement, both of which are necessary to outline in this paper before presenting my findings.

The prisoners' movement was nationwide, and it had a multitude of aims that included exposing abusive prison policies, calling for the eradication of the prison system's inhumane conditions, calling for organizing of prison labor unions and political power, and even calling for reductions in the overall prison population. It is important to note that the prisoners' rights movement did not occur in a vacuum, rather it was a continuation of organizing that was occurring on the outside of prisons, such as the Civil Rights Movement, the Anti-War movement, and the Black Liberation Movement to name a few. An important figure that rose to prominence out of this movement and with the help of the Black Panther Party for Self-Defense, is that of George Jackson, who arguably became face of the prisoners' movement. Jackson's prominence rose with his induction into the Black Panther Party for Self-Defense and his writings about race, revolutionary political thought, and the injustices of the prison system. His book

Soledad Brother: The Prison Letters of George Jackson was released in 1971 selling 400,000 copies internationally. Along with his influential writings, Jackson was also instrumental in organizing and politicizing those on the inside, and during his incarceration he became a part of a case that was heavily documented by the media—the case of the Soledad Brothers.

George L. Jackson, Fleeta Drumgo and John W. Clutchette became known as the Soledad Brothers, their case catching the Nation's attention and turning heads to look deeper into the United States' prisons, when the three men were falsely accused of murdering a prison guard in retaliation for the murder of three prisoners at Soledad Prison in January of 1970. The Soledad Brothers Defense Committee was created with support of many people on the outside including Angela Davis, a well-known professor at the University of California, Los Angeles at the time. The Soledad Brother's case received its height of visibility on August 7th, 1970 when Jonathan Jackson, the younger brother of George Jackson took over the Marin County Courthouse, taking hostages in exchange for the Soledad Brothers freedom. Jonathan Jackson and all but one hostage in his possession were killed by police on the scene only moments after leaving the courthouse. Ruchell Magee, one of the prisoners that joined in on the takeover, was the only survivor. Following the incident, Angela Davis was indicted and imprisoned for conspiracy in the takeover when it was revealed that the guns used in the takeover were registered under her name. The national coverage that the Soledad Brothers were receiving skyrocketed after the Marin Courthouse takeover, drawing the nation's attention to the injustices of the criminal justice system.

On August 21, 1971 only a year after the Soledad Brothers case caught the public's attention and a few weeks before the official trial was set to begin, George Jackson was shot to death by

prison guards at San Quentin State Prison in an alleged escape attempt. It was ruled out as justifiable homicide, but the details of that day still remain unclear, or as unclear as the dominant narrative portrays it. The repeated story is that George Jackson after meeting with attorney Stephen Bingham snuck a gun into San Quentin Prison and attempted to escape along with six other prisoners. The six prisoners, Luis Talamantez, Hugo Pinell, John Larry Spain, David Johnson and Willie Tate were put on trial thereafter, and became known as the San Quentin Six. The evidence supporting claims of an escape attempt have proven dubious if even practical over the years, and many activists and organizers insist that the alleged escape was a frame-up created to justify Jackson's murder.

Figure 1 illustrates the connections that each of the San Quentin Six had to prison activism prior to George Jackson's murder and the indictment of the San Quentin Six. As seen in the flowchart, each of the San Quentin Six and Ruchell Magee had filed affidavits against Soledad and San Quentin Prison for murders of prisoners W.L. Nolen, Cleveland Edwards, Alvin Miller and Fred Billingsea. The flowchart not only serves as a visual representation of the connections between the multiple cases, but it is also reverse engineers exactly how the state managed to discredit prison activists and distort the narrative surrounding the movement.

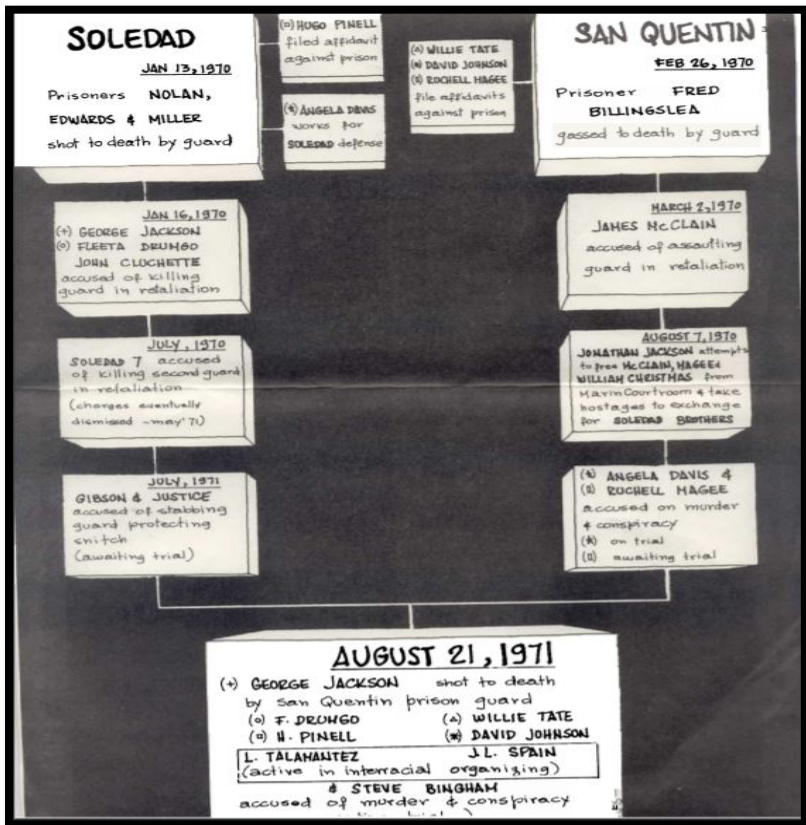


Figure 1. A flow-chart distributed during the San Quentin Six Trial, located at *ItsAboutTimeBPP* Archives in Sacramento, California and the *Freedom Archives* in San Francisco, California.

Methods

In order to investigate the declension narrative and systemic repression stemming from the movement, this project uses archival research methods by analyzing primary and secondary

sources. Primary sources include George Jackson's *Soledad Brother* and *The Blood in My Eye*, Angela Y. Davis *If They Come in the Morning* and a plethora of materials (documents, audio interviews and footage) from the *Freedom Archives*, the official archive of the Black Panther Party *ItsAboutTimeBPP* and a personal archive that I gained direct access to. This personal archive includes documents that were either collected by the archiver or donated, and as a part of this project I am digitizing and scanning materials from the archive to make it available online to the public in the near future. These primary sources were used for content analysis and to better understand the perspectives and objectives of prison organizers and activists during the movement, while secondary sources such as Heather Ann Thompson's *Blood in the Water*, Dan Berger's *Captive Nation* and Eric Mann's *Comrade George: An Investigation into the Life, Political Thought, and Assassination of George Jackson* were used to frame the movement and explore the research that has already been completed in the past.

Oral History

A large aim of this project as aforementioned, is to document oral histories with goals of preserving histories that have been obscured or looked over by the declension narrative. To conduct this portion, I utilized a traditional oral history method in which I conducted interviews with survivors and participants of the movement. These interviews sought not just to receive information about the movement from a historical perspective, but to identify each subject's relationship to that history by tracing events of their life and each period of their life. Some questions include *When and where were you born?* or *What was your upbringing like?* I chose the oral history method so that each interviewee's relationship to the movement would become present and any gaps in the narrative that has been constructed around the movement could be further explained.

For instance, in my interview with John W. Clutchette, the only surviving Soledad Brother, I was able to trace the trajectory of his life and better understand exactly why tenets of the Black Panther Party for Self Defense appealed to him more so than other politically active groups present at the time. In my interview with Karen Wald, a journalist and member of the Soledad Brother's Defense Committee, her connection to activism became apparent as she spoke about her experience with student and anti-Vietnam War movements prior to her involvements in the prisoners' rights movement. The oral history interviews seek the facts and truths that can be corroborated through documentation and evidence, but still based on the perspective and life experience of the interviewee.

Findings

The declension narrative present within discussions of the prisoners' rights movement does not stand alone in the creation of an unnuanced and non-cyclical narrative. Further purposeful distortions have contributed greatly to the current dominant narrative and relates to the backlash and increased systemic repression within prisons and the criminal justice system. The following findings represent both categories of a distorted and deteriorated narrative, and each has a different impact on the legacy of the prisoners' rights movement and the prison system.

Increased Censorship inside Prisons

Throughout the 1960s, courts across the nation saw an influx of cases drawing attention to the infringements upon prisoners' rights, and more specifically the right to practice freedom of religion and freedom of speech. The Nation of Islam, a radical religious group formed in the 1930s, was at its height and the organization's ideology was widespread and heavily studied by many Afro-American prisoners who spearheaded the fight for prisoners' Constitutional rights. Among those fights, were

several cases urging for Afro-American Muslims “freedom from punishment on account of one’s religion, the opportunity to hold religious service, and the right to wear religious medals”³ as well as guaranteed access to the Qur’an, newspapers such as *Muhammad Speaks* and correspondence with their religious leaders. In 1964 the Nation of Islam broke ground for prisoners’ rights with the Supreme Court ruling for *Cooper v. Pate* which ruled that prisoners have the lawful standing to address their grievances before a court under the Civil Rights Act of 1871, noting that the Supreme Court is obligated to acknowledge the constitutional rights of prisoners (*Cooper v. Pate*, 378 U.S. 546, 1964). This case was cited in over 200 other court cases during the period that served to increase prisoners’ access to outside literature, legal counsel, and end to inhumane and brutal prison policies. Most notably, this decade of legal pursuit gave way to the radicalization and politicization of prisoners through access to books that were not only related to the Nation of Islam, but other political organizations like the Black Panther Party for Self-Defense. George Jackson, who himself had been incarcerated since 1960, benefited greatly from this change. If it were not for the success of *Cooper v. Pate*, letters written by Jackson between 1964 to 1970 would have never been published in his book *Soledad Brother* that served to voice Jackson’s politics and knowledge that was inspired by works from Mao Zedong and Kwame Nkrumah. In my interview with John W. Clutchette, he speaks to the importance of literature and reading groups for politically charged prisoners:

“Everybody read. I don’t care what kind of book it was, you had to read and study. And you couldn’t pretend that you read because we would have book gatherings and the brothers would know if you had read or not. But like I say it was a self-

³ Colley, Zoe “All America Is a Prison”: The Nation of Islam and the Politicization of African American Prisoners, 1955–1965” (2014) 393-415

serving because like I say, its hard to educate a fool, its hard to talk to a fool, its hard to reason with a fool. So you don't go down to brothers level, you bring 'em up to yours."

Interview with John Wesley Clutchette, 7 April 2019

This mentality that Clutchette describes served as the basis for organizers like George Jackson in his pursuit of educating himself and others. Louis Sander Nelson, the warden of San Quentin State Prison at the time of George Jackson's assassination, begged to differ in an interview given after George Jackson's death when asked why California State Prisons gave prisoners access to revolutionary material, stating: "if we put a man in a cell, and by nature of his acting out we require to keep him in his cell then we feed him all the revolutionary works, and George Jackson had at least a hundred or more revolutionary works, then of course he's not concerned with bettering himself, he's only looking forward to the day when he can get out a become a true revolutionary."⁴ This quote was not very far removed from the still underway investigation into the details surrounding George Jackson's death, and the outrage and prison uprisings that followed was enough to make the state error on the side of caution in relation to prison organizing.

After the decline of the movement, censorship processes changed drastically and appeared to take a major step backward. In 1989, the Supreme Court ruled in *Thornburgh v. Abott* that prisons could not ban access to all books, granting it unconstitutional under the First Amendment, however it granted "broad discretion" in examination for inappropriate content deemed "detrimental to the security, good order, or

⁴ This quote was taken from an interview that was first shown on August 23rd, 1971. The entirety of the news clip is held at Fremantle Media Archives located in London, UK. The footage includes exclusive interviews with the warden of San Quentin, George Jackson's mother, and Angela Davis.

discipline of the institution or if it might facilitate criminal activity” (Thornburgh v. Abbott, 490 U.S. 401 (1989)). Since the ruling, the type of books banned in prisons has increased from institution to institution. In my interview with Gail Shaw, an organizer during the period and close friend of the Jackson family, she explains that along with this increased repression through censorship, prisons across the nation have since seen a dramatic decline in the upkeep and presence of prison libraries.

Only two and a half decades after the groundbreaking *Cooper v. Pate* decision, *Thornburgh v. Abbott* sparked a major change in the leniency of institutions allowing in radical materials. The rhetoric utilized in the ruling, claiming that if materials appeared to pose a threat to security of the institution, allowed institutions to prohibit political materials. Despite *Abbott’s* clause claiming that censorship cannot be “solely because its content is religious, philosophical, political, social or sexual, or because its content is unpopular or repugnant”⁵ several lists of banned books throughout the nation appear to contain the exact content.

Discreditation of Prison Activists

Amid the decline of the prisoners’ rights movement, a major attempt to link prison organizing and activism to gang activity was seen with entities such as the Federal Bureau of Investigation’s counterintelligence program (COINTELPRO) that sought to target, surveil, infiltrate, neutralize and discredit domestic political organizations. Following the assassination of George Jackson, the FBI worked to tie Jackson to the notorious prison gang the Black Guerilla Family (BGF). In my interview with John W. Clutchette, he mentions that widespread knowledge of the BGF did not come about until the 1980s

⁵ See Thornburgh v. Abbott, 490 U.S. 401 (1989) U.S. Supreme Court syllabus and documentation

while he was incarcerated, and that Jackson himself never mentioned the gang and was opposed to gangs by nature. Clutchette himself was still accused of being a member of the gang, even up until his most recent parole hearings in which Governor Brown used his alleged membership of the gang as reason for denying Clutchette's parole. Moreover, on the FBI's public records site, all documents pertaining to the BGF and surveillance of the organization do not date until 1973, two years after Jackson was assassinated, yet he is still recognized as the gang's founder. The documents claim that prisoners who were apart of the gang credited Jackson as the founder, but throughout this period "many prisoners were pushed to testify against the Soledad Brothers"⁶ with false testimonies to incriminate the Soledad Brothers and more specifically George Jackson, a practice that did not end following his death.

The Shape of Political Imprisonment in the United States and the Decline of its Visibility

Throughout the movement, there was an emphasis on highlighting the cases of political prisoners by activists despite the United States concealment thereof, and refusal to acknowledge its existence. According to the first amendment of the United States Constitution, freedom of speech, religion and expression are guaranteed inalienable rights that are protected by the government. The amendment states that "Congress shall make no law respecting an establishment of religion, or prohibiting the free exercise thereof; or abridging the freedom of speech, or of the press; or the right of the people peaceably to assemble, and to petition the Government for a redress of grievances."⁷ This freedom of expression clause is often suggested as evidence to the United States' lack of political prisoners or imprisonment of people for political activities that seek to petition the government and its

⁶ Mann, Eric. *Comrade George*. New York: Harper & Row, 1974.

⁷ U.S. Constitution Amend. 1.

ideologies. Prisoner activists during the movement emphasized the danger in defining political imprisonment in a nation through the lens of those “controlling the ongoing war” (Dutch and Susler). Michael E. Deutsch and Jan Susler’s paper titled *Political Prisoners in the United States : The Hidden Reality* complicates the theoretical and practical framework of political imprisonment in the United States by dividing the definition into three distinct categories: “(1) foreign nationals whose political status or political activities against allies of U.S. imperialism result in detention of imprisonment; (2) members of U.S. oppressed nationalities (African-Americans, Puerto Ricans, Chicanos/Mexicanos and Native Americans) who are prosecuted and imprisoned for political activities in furtherance of their movements for liberation and justice; [and] (3) white people who have acted in solidarity with the liberation movements of the oppressed nationalities and/or in opposition to U.S. foreign or domestic policies. A key component of these distinctions that is defined within the members of U.S. oppressed nationalities sector, as defined by Deutsch and Susler are the prisoners of war--who have participated in “armed struggle.”⁸

In my interview with Bill Jennings, a former Black Panther of the Oakland chapter, Jennings mentioned that over a hundred Panthers were in prison at one time across the country. The official reasoning behind their imprisonment however was never solely political, and government documentation often attributed their imprisonment to murders of prison guards and police officers. Angela Y. Davis’ book, *If they come in the morning* further speaks to this phenomenon of political imprisonment in the United States stating that “the political prisoner’s words or deeds have in one form or another embodied political protests against the established order and

⁸ Deutsch, Michael E., and Jan Susler. "Political Prisoners in the United States: The Hidden Reality." *Social Justice* 18, no. 3 (45) (1991): 92-106.

have consequently brought him into acute conflict with the state. In light of the political content of his act, the “crime” (which may or may not have been committed) assumes a minor importance. In this country [...] the political prisoner inevitably stands trial for a specific criminal offense, not for a political act. Often the so-called crime does not even have a nominal existence.”⁹ The emphasis on material regarding the nature of political imprisonment and the cases of political prisoners has shifted dramatically since the decline of the movement. Much of this can be related to the distortion and declension narrative surrounding the movement and incarceration today.

Conclusion

While there has been an increasing momentum towards re-evaluating the criminal justice system and the system of carceral punishment in the United States, much of this movement has developed without the benefit of an understanding of its roots in the prisoners’ rights movement and prisoner activism of the 1970s. As shown throughout this paper, much of this history has been either repressed or buried and lie in boxes of archives, and in the unrecorded memories of key participants of this movement. This history should be re-examined and brought to the surface so those who challenge the prison system today can learn from its successes and mistakes.

References

Berger, Dan. *Captive Nation: Black Prison Organizing in the Civil Rights Era*, 2014.

⁹ Davis, Angela Y. *If They Come in the Morning: Voices of Resistance*. New York: Third Press, 1971.

Chase, Robert T. 2015. "We Are Not Slaves: Rethinking the Rise of Carceral States through the

Lens of the Prisoners' Rights Movement." *Journal of American History* 102 (1): 73–86.

Clutchette, John W. interview Oakland, Californian April 7, 2019

Colley, Zoe "All America Is a Prison": The Nation of Islam and the Politicization of African American Prisoners, 1955–1965" (2014) 393-415

Davis, Angela Y. *If They Come in the Morning: Voices of Resistance*. New York: Third Press, 1971.

Deutsch, Michael E., and Jan Susler. "Political Prisoners in the United States: The Hidden

Reality." *Social Justice* 18, no. 3 (45) (1991): 92-106.

Footage of Warden Louis Sander Nelson, August 1971, Fremantle Archives, 1 Stephen Street London W1T 1AL, United Kingdom

Jennings, Bill interview Sacramento, California June 23, 2019.

Mann, Eric. *Comrade George*. New York: Harper & Row, 1974.

Process Editors. *Organizing the Prisons in the 1960s and 1970s: Part One, Building Movements*, 2016.

Shaw, Gail interview Sacramento, California June 23, 2019.

Thompson, Heather Ann. *Blood in the Water: The Attica Prison Uprising of 1971 and Its Legacy*. First ed. 2016.

Wald, Karen interview Oakland, California August 18, 2019.