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Maternal Immune Activation (MIA) in Mice: A Study to Phenotype ASD-Related Communication Behaviors and Analyze Maternal Health Outcomes in the US

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Maternal Immune Activation (MIA) in Mice: A Study to Phenotype ASD-Related Communication Behaviors and Analyze Maternal Health Outcomes in the US

Honors Thesis

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PART I: LITERARY ANALYSIS OF MATERNAL HEALTH OUTCOMES IN AMERICA

Note: While the present animal study analyzed the behavioral and communicative deficits found in offspring as a function of maternal immune activation, the focus of this literary analysis is on the general politics surrounding maternal health within the US and is a reflection of my personal interests in this area of study.

INTRODUCTION TO MATERNAL HEALTH IN THE US

Normally, when we think of the United States we think of phrases like "land of the free", and "home of the brave", a place where dreams really do come true and the American dream becomes a reality. This is a nation often seen as a gold standard of comfort, health, and lifestyle, our poor standards of maternal healthcare little known to the global community. In fact, the US ranks as the worst developed nation in the entire world for maternal health (IHME 2015). As global maternal mortality rates fell by over one third from 2000 to 2015, leading to one of the greatest triumphs in global health, America's rates spiked (UNICEF 2017). While this is very staggering in and of itself, even more alarming is the notion that most people aren't even aware this is the case and as a result, the state of maternal healthcare is not improving, but instead is slowly and silently declining.

UNICEF refers to maternal mortality strictly as deaths due to complications in pregnancy or childbirth (UNICEF 2017). In a recent study conducted by the World Health Organization (WHO), it was found that the maternal mortality ratio for America more than doubled from 12 to 28 deaths per 100,000 births between the years 1990 and 2013 (WHO 2014). According to the US Agency for Healthcare Research and Quality, 60 billion USD were put into maternal care in 2012 (AHRQ 2015). Despite the funding put into this area of

healthcare, it is estimated that each year, 1200 women suffer pregnancy and childbirth-related complications that prove to be fatal and an additional 60,000 face near-fatal complications (WHO 2014; Creanga et al 2014).

In 1986, the Centers for Disease Control and Prevention (CDC) began a national surveillance of pregnancy-related deaths in the US in an effort to delineate the causes of maternal death and its rising rate. Each year, the 52 reporting areas – the 50 states, NYC, and Washington DC – are requested to voluntarily send death certificates for those women who lost their lives during pregnancy or within 1 year of pregnancy in those cases where this information is available (CDC, 2017). Several factors have contributed to America's startling statistics on mortality with each passing year.

GENERAL RISK FACTORS AND PREVENTION OF ADVERSE OUTCOMES

The CDC found that during the years 2011-2013, the top ten causes of maternal death in order were cardiovascular diseases, non-cardiovascular diseases, infection/sepsis, hemorrhage, cardiomyopathy, thrombotic pulmonary embolism, hypertensive disorders of pregnancy, cerebrovascular accidents, amniotic fluid embolism, and anesthesia complications (CDC, 2017). Almost all deaths due to pregnancy and childbirth are preventable and yet the US continues to face obstacles in improving the state of maternal health. Many of these issues stem from poor personal health, often related to cardiovascular problems and high blood pressure. These issues are driven by an increase in poor diet and obesity in America, both of which are increasingly implicated in the many chronic health problems facing Americans today.

It seems that one not-so-simple fix may be of great use in this case – the promotion of better personal health practices. This is easier said than done, of course. But considering the fact that such a large number of women die each year due to complications that are entirely preventable had they lead healthier lives, it becomes a necessary measure to take in order to educate women about their own health. Regular exercise and healthy diets are the obvious recommendations for lifestyle changes related to optimal heart and body health. But many of the risks associated with diabetes, hypertension, diabetes, and a stressful lifestyle can also be prevented and "improved with pre-pregnancy healthcare" where preventative healthcare can go a long way according to Dan Frayne M.D. (Becker, 2016). This includes things like regular check-ups and increasing patient awareness and involvement in their own health.

Dr. Michael Brodman, chairman of the Department of Obstetrics, Gynecology and Reproductive Science at Mount Sinai reflected that the rising level of obesity in today's world means that physicians need to come up with specialized treatments because obese individuals represent a different kind of patient (Becker, 2016). One way to alleviate these problems might be to treat these pregnancies as high-risk from the start. When expecting mothers and physicians are aware of the chronic conditions affecting them, doctors may be better equipped to deal with high-risk situations that may arise, or implement measures of preventative care to eliminate such potential altogether. This might entail communication between primary care providers and professionals involved in prenatal care, and is a necessary step to take to improve health outcomes.

POVERTY, ACCESSIBILITY, AND THE POLITICS OF REPRODUCTION

In 1999, the CDC listed family planning as "one of 10 great public health achievements in the United States during the 20th century" (CDC, 1999). Yet, TRAP laws and new measures in motion to defund family planning services and abortion clinics, causes the burden of unwanted pregnancy to be placed entirely on women who are in equal parts terrified by the prospect of their lives with an unwanted child, and distrusting in a health and legal system that has failed to meet their needs.

Tsui et al. note that while it is difficult to confidently attribute the maternal mortality ratio to a single cause and identify a single solution, "evidence exists that meeting the need for family planning can reduce maternal mortality" (Tsui et al., 2010). Overwhelming evidence exists to support this notion as one might turn to Egypt as the epitome of successful family planning. The country's maternal mortality ratio decreased 50% between 1992 and 2000, a figure that is concurrent "with increased uptake of family planning and other maternal health improvements" (Tsui et al., 2010). Another study estimated that maternal deaths would be 19% higher without the use of contraception (Ross, 2009). Clearly, family planning and access to contraception plays a large role in the overall health of women – for both those who want and do not want pregnancies. However, within this seemingly obvious solution lays another problem – one of accessibility.

While a discussion of family planning and maternal health impacts the lives of all women, it has been overwhelmingly reported that poor women and women of color often face the vast majority of adverse health impacts. From this point of view, maternal mortality is just one facet of the many issues impacting overall maternal health in the US. The CDC has reported figures of pregnancy-related mortality from 2011-2013 indicating

that there were 12.1 deaths per 100,000 live births for white women. For women of other non-white and non-black races, this number was 16.4. For black women, the figure rose to 40.4 deaths per 100,000 live births (CDC, 2017). It begs the question then, where are the gaps in healthcare?

In one study, it was found that African American pregnant women "are nearly four times more likely to die from pregnancy-related complications than are white women" and are "two to three times more likely to experience preterm birth, and three times more likely to give birth to a low birth weight infant" (Collins et al., 2004). Most interestingly, such disparities were seen across various medical and socioeconomic risk factors, where income, level of education, and tobacco and alcohol use were all taken into consideration. In fact, the gap between maternal health outcomes for black and white women widened as socioeconomic levels rose (Kleinman, 1987). Researchers have come to explain these observations as a direct result of the stress induced in pregnant women of color by racism. The persistence of systemic racism in the fabric of American society "serves as a course of chronic stress, negatively affecting the body's hormonal levels, which can initiate physical mechanisms that may lead to preterm birth" and other adverse pregnancy-related outcomes (Collins et al., 2004).

With the issue of race and accessibility comes that of insurance and coverage. According to a recent survey commissioned by Childbirth Connection, women with insurance pay an average of $3,400 out of pocket for childbirth. This cost comes without factoring in pre- and post-natal care. When taking this into consideration the average price "for pregnancy and newborn care was about $30,000 for a vaginal delivery and $50,000 for

a C-section, with commercial insurers paying out an average of $18,329 and $27,866" (Truven, 2013).

For those women and families below the poverty line, these are unthinkable figures. The complications that may arise on account of women's predispositions and preexisting conditions mentioned earlier are yet another added cost not factored into the figures presented. For many of the women in question these costs act as a catalyst for chronic stress, mental illnesses like depression, and the associated physical conditions that accompany such conditions – hypertension, cardiovascular problems, and other factors that increase maternal health risks.

MODE OF DELIVERY AND ITS IMPACT

As the numbers indicate, birth by cesarean-section (C-section) incurs a much higher cost than vaginal delivery. Not only is it costlier in terms of finances, but it is also taxing in terms of physical health. A new Consumer Reports study found that the most common surgery performed in hospitals across the US has nothing to do with hearts or bones – but is rather a C-section. Nearly one in every three babies are delivered by cesarean section today (Haelle 2016). While C-sections are necessary in many cases, they are not and should not be the first line of action in delivering a baby. The rates of C-sections in America far exceed the benefits and medical necessity of this practice. For a practice that elevates costs and risks to mother and child, it's difficult to make sense of these increasing rates.

With the rising influence of an overzealous legal system and people looking to sue physicians comes the theory that doctors provide "the most aggressive care possible to avoid a negligence lawsuit" (Thielking 2015). A 2009 study found that states that have

higher caps on malpractice settlements see slightly higher rates of C-sections than those states with lower caps (Yang et al. 2011).

Still in other cases, women choose C-sections in an effort to exercise control over their childbirth than waiting for natural labor. It becomes like any other medical procedure with a choice of surgeon, handpicked date and time, and complete clarity on how the procedure will happen. While this is a factor, its influence on maternal health outcomes is limited because elective "C-sections account for just under ten percent of all of the scheduled procedures in the US" (Jun et al. 2010). With increasing awareness on the risks and over-reliance on C-sections as a delivery method, many hospitals are working to lower the rates and educate expecting mothers on the alternative, while optimal maternal health remains a priority.

GLOBAL COMPARISONS

As technological advancements have changed the face of medicine across the globe, we have seen a significant rise in instances of assisted fertility. A study at the University of Adelaide (Australia) recently found that the risk of significant birth complications like still and preterm birth, low birthweight, and neonatal death is nearly twice as high for babies conceived through assisted conception when compared with naturally conceived children (Marino, 2014). Currently, this is the most comprehensive study of its kind where researchers surveyed the outcomes of over 300,000 births in Australia over a period of 17 years. Of them, over 4300 were births of assisted reproduction including methods like in-vitro fertilization, tracytoplasmic sperm injection, ovulation induction, and cryopreservation (freezing of) embryos. The researchers found that assisted reproduction

cause babies to be twice as likely to be stillborn, nearly three times more likely to have low birth weight, twice as likely to die within the first month of life, and more than twice as likely to be preterm. In the context of America, assisted conception has been on the rise for decades now. In 2003, doctors performed nearly 113,000 cycles of IVF; in 2012, this number increased almost 50 percent to 165,000 cycles (NPR, 2014). Though methods like IVF help countless women who struggle with fertility, these figures are startling when combined with the increased risk factors to infant health. The researchers at the University of Adelaide believe that more research is needed to address more recent advancements in medicine and technology as they undergo constant innovation and change. It is likely that changes in medical technology and self-care strategies in more recent years will influence the associated risks.

While some changes are being affected in terms of preventative care and general health promotion, the question remains of why health outcomes are so strikingly different between the US and countries like Norway, Finland, and Iceland, all of which rank as the top three nations for maternal health outcomes, respectively (STC, 2015). Though it is widely known that American healthcare spending is unsurpassed, why is it that a mother and her unborn child in Finland or Sweden face more positive outcomes than those in the US? To take a cue from some Scandinavian models of healthcare would mean to adapt a more holistic approach to medical treatment. Looking at Sweden's Sexual and Reproductive Health and rights (SRHR) Agenda, four key strategies can be adopted as Sweden has done in its commitment to improve maternal and child health outcomes. The agenda is centered on strategic communication to improve education and policies on SRHR, allocating more resources to maternal healthcare services (including family planning and

safe abortions), increasing support and communication between health care providers (such as midwives and primary care providers), and improving access to SRHR-related information, decision-making, and education for women across the nation (Sida, 2011).

When looking further into the State of the World's Mothers report published by Save the Children, five important indicators are taken into consideration to derive the mothers' index. These factors are: maternal mortality rates, child mortality rates (under 5 years old), educational status, economic status, and political status. Many of these are not surprising factors considering their obvious relationship to health outcomes in general – such as education and economic status that contribute to an individual's accessibility to medical care. One factor that is often overlooked is political status, defined as "Participation of women in national government" and further seen as women having a voice in politics so "issues that are important to mothers and their children are more likely to surface on the national agenda and emerge as national priorities" (STC, 2015). The same study found that women hold less than 20 percent of all seats in the US Congress, where "nearly half of all countries in the world perform better on this indicator."

In Sweden, for example, women hold 44 percent of parliamentary seats and their collective voices are not lost on the deaf ears of lawmakers who do not have the capacity to relate to them. Sweden is also committed to improving health services for women in poverty through the Swedish International Development Cooperation Agency (Sida), which supports a project intended to reach women through the workplace in factories in low- and middle-income countries. According to Sida, the project has contributed to "a 60 percent increase in the number of prenatal medical visits during participants' most recent

pregnancy" in Mexico (Sida, 2011). Sida works to improve lives not only in Sweden, but also all across the globe.

It is important to note that standards of living vary greatly between European nations and the US, where obesity is far less common and commuting by foot or bicycle is the norm across many European cities. This in effect, might contribute to overall health outcomes, as would differences in levels of income inequality and stress levels between the two continents. In addition, our fee-for-service model changes the premise of incentives for American doctors, while Swedish doctors serve their communities as salaried employees. Perhaps these differences make it difficult to draw a true comparison between these countries but there is something to be said for the efficiency and increased positive outcomes that result from European models of healthcare. By treating maternal health as a topic of political importance, healthcare as a human right, and creating a system that ensures everyone has equal access to services and political representation, countries like Sweden can be taken as archetypes for increased positive health outcomes.

CONNECTION TO CURRENT STUDY

Though a great deal of this literary analysis shows that maternal health in the United States is greatly shrouded in startling facts and figures, there is optimism to be found within the medical community and the strides it is making to improve upon women's health. While there is fear that Obama-era policies might see drastic changes with the current administration, if the Affordable Care Act (ACA) remains intact, prenatal and maternal health will remain benefits that insurance plans must cover (WHO, 2015). Additionally, many states have lessened the economic hurdles that previously precluded

women from being able to access prenatal care by extending insurance coverage to low-income women through the ACA. As the data shows, a vast majority of maternal deaths and pregnancy-related complications are entirely preventable as are adverse outcomes that impact offspring. Therefore, numbers that represent increasing rates in adverse health outcomes for American women are numbers we can work to improve if we make maternal health and preventative care a priority.

It is evident that maternal stress is one such factor that is often concurrent with other, preventable health issues such as hypertension, cardiovascular disease, and risk of infection, all of which disproportionately affect women in poverty. Most notably, studies frequently link stress to impaired immune function. One study conducted at the Ohio State University College of Medicine found that students' immunity went down every year under the stress of a three-day final exam period (APA, 2006). Specifically, researchers found that test takers had fewer natural killer cells, immune cells that help fight tumors and viral infections. Put in the context of pregnancy, it is unsurprising that an increase in stressful conditions and impaired immune function is a detriment for both an expecting mother and her child, who are made more susceptible to outside factors and pathogens.

In fact, researchers at the Mailman School of Public Health at Columbia University recently found a trend that links influenza-like illness with an increased risk of ASD in offspring. Though their results were reported as being statistically insignificant, their data points to a significant trend that warrants further examination. They reported that "children born to mothers with laboratory-verified flu and matching symptoms had nearly double the odds of later being diagnosed with ASD compared to women without flu and symptoms" (Paul, 2017). Their findings are consistent with past research suggesting that

maternal viral infection during the first trimester and bacterial infection during the second is associated with increased ASD risk for offspring (Pineda, 2013). They did note however, that such infections likely contribute to increased risk on account of the body's natural inflammatory response when the maternal immune system is activated. The connection to ASD is more likely a result of this response rather than the flu or viral infection itself.

The present project aims to study some of these connections by analyzing the communicative deficits that arise in offspring as a result of Maternal Immune Activation (MIA) in mice, effectively implicating MIA as an environmental risk factor for ASD.

PART II: MATERNAL IMMUNE ACTIVATION STUDY

ABSTRACT

Core symptoms of Autism Spectrum Disorders (ASD) include deficits in social/communicative behaviors, and repetitive/stereotyped behaviors. Mouse models are a highly established paradigm used to study the phenotypic deficits that result from various inducible genotypic or environmental risk factors for ASD. Previous studies have demonstrated a link between maternal immune activation (MIA) and ASD-like behaviors in mouse models. In this model, the maternal immune system is activated during pregnancy by injecting the viral mimic poly(I:C). The resulting offspring are phenotyped and analyzed with regards to their communicative behaviors.

Previous studies have demonstrated that male pups born to dams with immune activation produce fewer ultrasonic vocalizations (USVs) in testing than do their saline-injected counterparts. It has also been found that MIA produces offspring with hallmark signs of ASD: social deficits and stereotyped, repetitive behaviors. In the present study, the MIA theory was assessed using a mouse model for ASD, and focused on the shapes of the USVs produced. Some significant differences were seen in call types between wild-type and MIA male animals that are indicative of differing levels of complexity in their communicative behaviors. These results provide insight into the impact of models like MIA as an environmental risk factor for autism and suggest a need for further research on ways to improve maternal health during pregnancy, as well as associated infant health outcomes.

INTRODUCTION

Currently, about 1 in 68 children in the United States are diagnosed with an autism spectrum disorder (Christensen 2016). Autism spectrum disorder (ASD) refers to a range of conditions of a neurodevelopmental disorder characterized by challenges in social skills, repetitive behaviors, and communication deficits. In 1977 Folstein and Rutter published a seminal study on autism, concluding that brain injury during the critical period in infancy, in combination with a potential genetic disposition, may be underlying causes for autism. Mouse models are increasingly being used to evaluate the unique roles of risk genes, epigenetic factors, and prenatal infections in ASD. Because there are no consistent biological markers for autism, current diagnoses are based solely on behavioral criteria (Pineda, 2013). Two core symptoms of autism are currently used to assess mice in behavioral testing: social abnormalities, and repetitive and stereotyped behaviors (Silverman, 2009; American Psychiatric Association, 2013).

Recently, maternal immune activation (MIA) was implicated as a possible environmental risk factor for ASD. The MIA model posits that immune activation during the first trimester of pregnancy can lead to developmental deficits in offspring. In a normal pregnancy, an elevated state of inflammation is seen in the mother and in the placenta. However, too much inflammation (as with MIA) can enhance risk of autism and schizophrenia in offspring (Pineda, 2013). MIA is thought to increase inflammatory cytokines in the fetal environment and fetal brain. In fact, adult autistic brain and cerebrospinal fluid (CSF) exhibit high levels of inflammatory cytokines.

In this paradigm, a pregnant mouse is injected with the viral mimic polyinosinic-polycytidilic acid (Poly(I:C)). Poly(I:C) is a human-made double-stranded RNA ligand that

has an affinity for Toll like receptor-3 (TLR3). TLR3 is highly involved in the immune response to viral infection. Several recent studies have been aimed at understanding the effects of TLR3 activation on brain function. Its effects are expressed as a spectrum of behaviors that can be induced by poly(I:C) and immune activation within the central nervous system (Cunningham et al., 2007). The advantage of this virus lies in the fact that the immune response that follows infection is limited to a 48-hour timeframe (Meyer et al., 2005). The efficacy of the virus can thus be quantified within the given window of time.

It follows that the time of injection during gestational period plays a key role in whether and how the virus might influence offspring behavior and mental health. Previous studies have shown that immune activation on gestational day 9 led to schizophrenia-like behavior in offspring, while MIA at later time periods like day E16-17 resulted in increased susceptibility to seizures (Pineda, 2013). This goes to show that the time of immune activation is highly relevant as both maternal and fetal immune responses vary through different periods of gestation (Tinsley, 2009). In a 2011 study by Natalia Malkova and her colleagues, it was found that MIA males show decreased sociability, and increased repetitive and stereotyped behavior as compared to their saline-injected, control counterparts, all hallmark signs of autism (Silverman, 2010).

In a pilot mouse study at the University of Connecticut, non-significant results were found, with some expected trends in behavioral differences seen between MIA mice and controls. In this prior work, several confounds (e.g., excessive maternal stress, inability to determine gestational age at time of injection, and incorrect viral dosage) limited the ability to draw definitive conclusions. However, In the present study, the impact of early prenatal viral exposure was measured under low-stress conditions, with precise determination of

first trimester gestation in order to determine whether MIA presents an environmental risk factor for autism. As in other models for autism, MIA is tested in mice using tasks that display characteristics of the core symptoms of ASD.

Previous studies in other labs have shown mice treated following this model were deficient in a battery of behavioral tasks used to phenotype mice (Naviaux et al., 2014). But they present a particular deficiency in ultrasound vocalization that measures communication skills (Malkova et al., 2012). This is indicative of the differential task-specific results associated with the MIA model. This potential specificity makes it an important and unique candidate for a study of autism, especially with regards to communication behaviors.

The specific goal of this project is to analyze the structural features of ultrasonic vocalizations (USVs) from mice born to dams exposed to Poly I:C, and from control mice born to dams that received saline. It was expected that some differences in number or complexity of different call types would be observed between groups. The vocalization task was run in addition to a battery of behavioral tasks typically used to phenotype behavior in mice including: pitch discrimination and ultrasound vocalizations, which measure auditory processing; the tube task, 3-chambered social interaction task, open field task, and elevated plus maze which measure sociability, dominance, and anxiety; and marble burying, which measures repetitive behaviors. A Rotarod task will show motor deficits, all characteristic of ASD. Although this complete battery of tasks was run and is noted in the methods section, the focus of the current analysis and results is on USV analysis. Overall, the current project has implications for the evaluation and screening of prenatal risk factors for developmental

disorders, and can be used in future studies to further research ways to reverse adverse effects and understand the impact of maternal health conditions on development.

METHODS

This project was designed through collaboration with both the Fitch Lab in the Psychological Sciences department (undertaking the responsibility for behavioral testing and phenotyping) and Dr. Mike O'Neill's lab in the Molecular and Cell Biology department (responsible for providing MIA induced animals for testing, and post-testing molecular analyses). Through the Fitch lab, a series of tasks was run to assess mouse behavior in both MIA and control models and analyzed for trends associated with autism. A preliminary behavioral study of the immune activation model was conducted in August 2016 (through the Fitch and O'Neill labs), however, the study found inconclusive data. In the current study, these tasks were replicated, and for the purposes of this thesis, special attention will be given to the results of the vocalization recordings. A brief summary of subject generation and other behavioral tasks performed on them is provided below.

Subject Generation

Female mice were obtained and housed under standard laboratory conditions. Mice were mated overnight. The appearance of a vaginal plug allowed for the marking of that day as embryonic day .5 (Malkova et al., 2012). The pregnant females were undisturbed until day 10.5, when they were weighed and randomly assigned to one of two groups – poly(I:C) or saline. Each group was comprised of five or six females. The test group was given intraperitoneal injections of given intraperitoneal injections of 5 mg/kg poly(I:C)

(potassium salt; P9582; Sigma, St. Louis, MO). The control group was injected with saline solution (5 ug^{-1}). Both groups were injected on day 12.5. The test condition did not have an impact on litter size; each litter was ~7-8 pups. Most pups were born around gestational day 20. The Fitch Lab initially received a total of 43 animals and testing began after they were weaned at 3-4 weeks of age. Of them, one control subject passed away, leaving the team with 21 test subjects, and 21 controls.

General Battery of Tasks and Hypothesis

1. Pitch discrimination: ASD models have shown evidence of enhanced pitch discrimination.
2. Marble burying: 20 marbles are placed in the testing area, the mouse is allowed in for 30-45 minutes; ASD models often display a high level of marble burying (repetitive action).
3. Open field: leave mouse in an open environment without obstacles to assess anxiety and exploration; ASD models should show decreased exploration.
4. Rotarod: place mouse on a rotating rod for 2 minutes as rpm increases to assess motor ability; motor deficits are often seen in ASD models.
5. Tube task: place experimental mouse on one side of a tube, add control mouse to opposite side; when one mouse steps out of the tube, results are recorded; ASD models generally show social anomalies (increased or decreased dominance) as measured by which mouse exits the tube first.

6. Elevated plus maze: used to measure anxiety by placing animals on a cross-shaped platform, where there is an open and enclosed space; ASD models show increased anxiety and tend not to explore open spaces.
7. 3-chamber social interaction: place a mouse in a 3-chambered box – 1 chamber contains a mouse, 1 contains a toy; allow subject in chambers for 10 min; the toy is then replaced by a second mouse for 10 minutes; the subject should show increased curiosity for the novel mouse but ASD models are likely to show deficits in sociability and spend more time with the toy.

Analysis of audio recordings

After the completion of behavioral tasks as described above, subjects were run on a USV test. USVs were detected automatically using an ultrasonic microphone (B&K) and acoustic analysis software (Adobe Audition). For each trial, female bedding was spread on the bottom of a clean cage and male test subjects were placed in that cage for a habituation period. Then, a female mouse was introduced into the cage. It was expected that the male would vocalize to the female using a variety of established mating calls, and that female vocalizations would be negligible (thus vocalizations recorded and analyzed are male-only) (Yang et al., 2013). Recordings were taken over 5 minutes, and vocalizations were automatically logged through our computer program. Using previously established call types, the vocal repertoire of each mouse was individually and manually analyzed for shape and duration into categories developed by Heckman et al (2016).

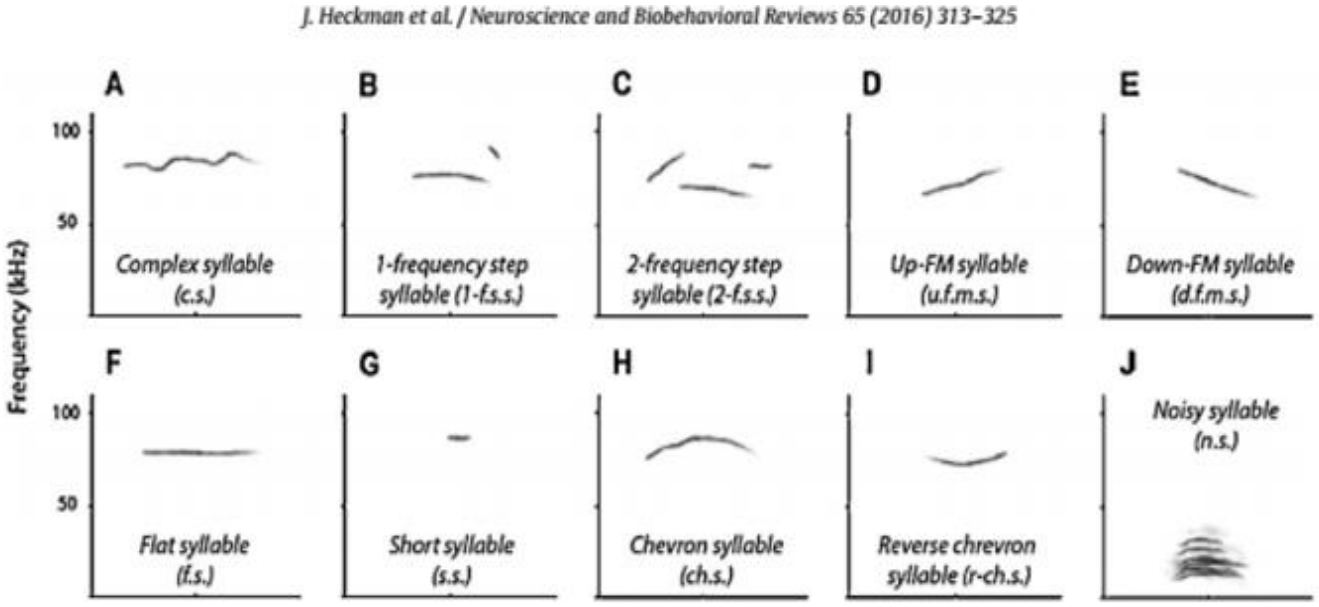

Fig. 1. Mouse USV subtypes, adapted from Heckman et al. (2016). In the current study, B and C were defined as a single category of "Frequency Jumps" and J was defined as "unstructured". An additional category called "modulated" was also included. The final ten categories used to analyze and label USVs were therefore: short, flat, up, down, modulated, complex, unstructured, frequency jumps, chevron, and reverse chevron.

RESULTS

A test of between-subject effects (SPSS, ANOVA) found a significant in total calls made difference between wild-type and MIA animals ($F [1,42] = 4.306$, $p = .044$). This shows that MIA has a measurable and meaningful impact on communicative behaviors, as would be expected in an ASD model. The following graph shows the more specific impact MIA has on mice with regards to call type and number of calls. Specifically, t-tests revealed a reduction in down and unstructured calls.

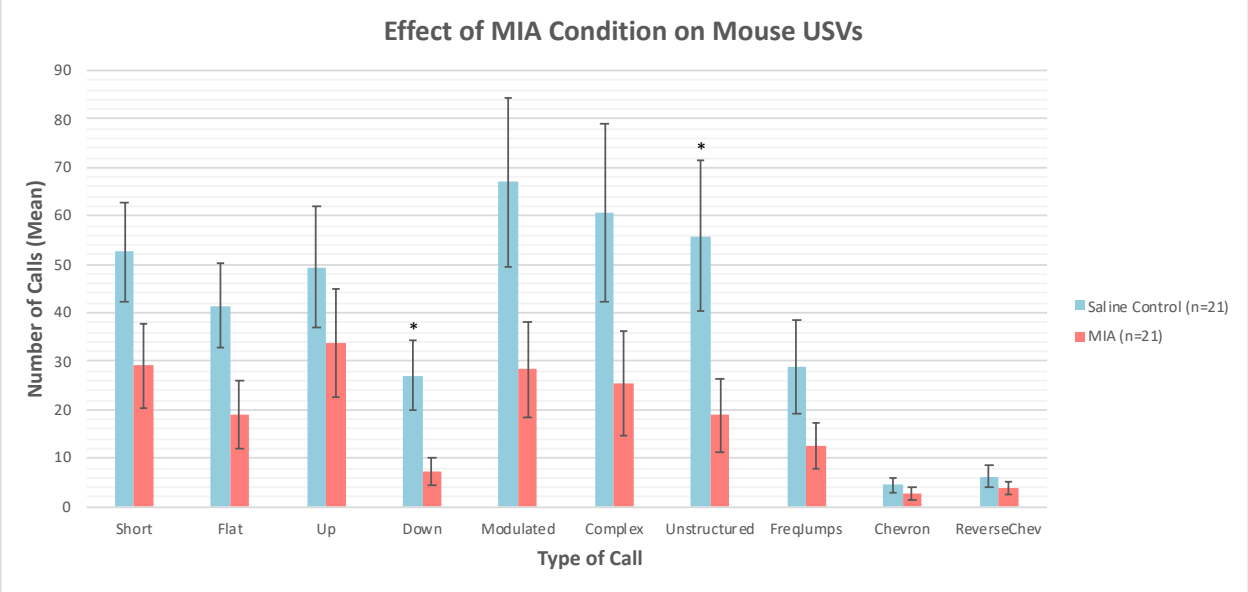

Figure 2. Effect of MIA Condition on Mouse USVs using ANOVA test. Condition 1 represents wild-type mice that were treated with saline solution. Condition 2 represents mice born from MIA mothers. The star (*) symbol demarcates conditions where a significant difference was observed between wild-type and MIA animals. The unstructured ($p=.04$) and down ($p=.014$) call types were seen significantly more in wild-type mice, showing that MIA has some degree of effect on communication in mice.

DISCUSSION

It is widely known that ASD is related to deficits in communication – both in mice and in humans. In this case, deficits were measured through an analysis of USVs. The results of this study indicate that the test condition had an effect on the overall differences between the MIA and control conditions, with significant differences in certain categories of mouse calls. In all cases, MIA mice vocalized less frequently on average than did control mice (Fig. 2). This is consistent with the findings of prior studies that have implicated maternal immune activation as an environmental risk factor for autism (Malkova et al., 2012).

According to one animal study, adult mice produce USVs in several social contexts (Portfors, 2007). The most widely studied context is male encounters with females (or in this case, female bedding), which induces male USV production (White et al., 1998). Socially, this is an important form of communication that functions to convey a male's attempt to engage the female's attention and/or attract a mate (Malkova et al., 2012).

In the context of humans, the American Speech-Language-Hearing Association has noted that ASD-related communication deficits often include impairments in joint attention, social reciprocity, and challenges in verbal and nonverbal communicative behaviors for general social interaction (ASHA, 2018). In this study, and in previously studied social paradigms, this translates to a decrease in the total number of vocalizations emitted by offspring that show ASD-related symptoms. As such, the MIA model is implicated in producing such outcomes.

In our study, differences between control subjects and subjects that came from MIA mothers were significant for the USV categories "unstructured" and "down." Control mice

emitted significantly more of these call types than did their MIA counterparts. It is worth noting that the "unstructured" label here is referenced as "noisy" in other mouse USV studies (Heckman et al., 2016). It is possible that in following with the general trend of wild-type mice vocalizing more, they produced calls that are also more complex in nature than mice born to MIA mothers. Unstructured calls tend to be more difficult to categorize manually because they contain elements of various call type structures without being pure tones, and are complicated by virtue of this lack of definitive structure. Because MIA mice vocalize less overall and this is an ASD model associated with communication deficits, it follows that they might also possess a less complicated vocal repertoire than controls (Malkova et al., 2012).

However, in general, the exact social meaning of the different call types is not well understood (Heckman et al., 2017). This makes the differences observed in these two particular categories of significant interest. Future studies should focus on why MIA mice used as an ASD model might show specific reductions in these types of vocalizations. Though we hypothesize this could be due to the higher complexity of these call types, confirmation would require further study.

CONCLUSION AND FUTURE DIRECTIONS

Through the course of this study, it was interesting to see how our current knowledge of neuroscience and animal models fits into the broader context of human health. Conducting a literary review provided a window into general maternal health outcomes both in the US and abroad, wherein it became clear that while adverse outcomes are rampant in America, they are also preventable and reversible.

Perhaps most interestingly, recent studies have shown that reversal of autism-like behaviors in adult mice is possible. One group of researchers used the MIA model to study the effects of a single dose of an antipurinergic drug – suramin – on the behavior and metabolism of adult mice. It was found that deficits in social behavior and metabolism are not permanent and can be reversed with antipurinergic therapy (APT) in the MIA model for ASD (Naviaux et al., 2014). They showed that purine metabolism is a regulator of behavior and metabolism and that a single dose of APT surgamin has the acute ability to reverse abnormalities – even in adult mice. However, in this study the mice received one dose of suramin, whose effects were lost after about 5 weeks of drug washout. Researchers have advised against the use of antipurinergic agents in humans because of the potentially toxic side effects that may occur with chronic treatment (Voogd, 1993). Further research on alternative drugs and clinical trials are necessary to study potential human outcomes.

Nevertheless, studies like these and the current results of our MIA project may lead to further research that could inform minimization of prenatal factors that increase ASD risk for offspring. For example, additional studies on the critical parameters (timing, degree, key inflammatory factors) associated with maternal immune activation and resulting behavioral deficits could inform screening and/or preventative measures during pregnancy. In addition, further study of the specific deficits observed in certain categories of vocalizations should be used to assess relevance to communicative aspects of ASD in general, for example by ascertaining whether similar specific patterns of vocalization anomalies are seen in other mouse ASD models, and by conducting further assessment on the particular social meaning of these particular calls. Such studies could substantially enhance our understanding of the communicative aspects of ASD in humans.

The present study and many other mouse models and animal trials also neglect to analyze the same behaviors in female offspring. Autism and its spectrum of disorders are widely known to impact human males more greatly than females but continued research is needed to advance the effectiveness of care and potential treatment of ASD for all people (Christensen, 2016). If it is known that sex differences do exist in rates of autism, it is possible that these differences go so far as behavioral phenotypes as well. It would be interesting to study these differences within the MIA model for ASD.

In general, studies such as this implicate stress and impaired immune function as factors in inducing adverse health outcomes (Collins et al. 2004). These results might be used to inform strategies for minimizing prenatal stress and the creation of healthier prenatal environments. It is evident that there is a need to bring maternal health to the forefront of research and policy work if we are to see improvements in human health outcomes. The only way forward is to lead with awareness and optimism grounded in science and continue working to ensure maternal health needs are met with care and caution through policy reform, education, and medical practice.

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