The Impact of Maternal Immune Activation on Social Cognition in a Mouse Model of Neurodevelopmental Disorders

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A Thesis

Presented to the Faculty of Mount Holyoke College

in partial fulfillment of the requirements

for the degree of Bachelor of Arts

with Honors

ABSTRACT

Many neurodevelopmental disorders like Autism and Schizophrenia are characterized by pervasive social behavior deficits, etiologically explained through both genetic and environmental factors. Epidemiological reports have linked maternal exposure to high- grade fever or viral infection during the second and third trimesters of pregnancy with increased risk of having a child later diagnosed with a neurodevelopmental disorder, including Autism and Schizophrenia. It is thought that in-utero exposure to an elevated maternal immune response, rather than a specific pathogen is likely mediating the increased risk of these disorders. Murine models of maternal immune activation (MIA) use Poly I:C (polyinosinic–polycytidylic acid), a viral mimic that is a toll like receptor-3 agonist, to induce an elevated immune response in the mother resulting in deficits in social approach behavior in the offspring. However, neural processes contributing to these social behavior deficits are unknown and may be linked to alterations in social cognitive development, suggesting that MIA might mediate deficits in social recognition as well. To test this hypothesis, this study utilized the Poly I:C MIA model in C57 Bl/ 6J mice to study the effects of maternal immune activation on social recognition behavior in offspring.

Pregnant dams were intraperitoneally injected with a single dose of either 20mg/kg Poly I:C or saline on gestational day 12.5; offspring were weaned on post-natal day (PND) 21 and a social recognition behavioral test was conducted at

PND 30, and again at PND 60. Typically developing control offspring from saline-treated dams showed robust social recognition during the juvenile period and this social memory was maintained in saline offspring when re-tested at PND 60. Conversely, offspring of Poly I:C-treated dams were found to show no preference between novel and littermate mice at PND30, indicating deficits in recognizing novel versus familiar social stimuli, and spent more time with the familiar littermate mice at PND 60. These data suggest that maternal immune activation mediates a delay in social cognitive and social recognition abilities and support the notion that cognitive and recognition deficits might be impacting the social behavior deficits observed in neurodevelopmental disorders like Autism and Schizophrenia.

ACKNOWLEDGMENTS

"My candle burns at both ends;
It will not last the night;
But ah, my foes, and oh, my friends—
It gives a lovely light!"

- Edna St. Vincent Millay

It's extremely hard to explain gratitude in words, but I guess I'll give it a shot…

To **Jared Schwartzer**, without whom this would have never been possible. Thank you- for every encouraging word, reality check, every moment of understanding, the freedom to explore, and the belief and trust you placed in me. I am extremely honored to have had the opportunity to work with you, and just want to let you know- you're my idol. I promise to take you up on those Saxophone lessons.

To **Geoffrey Tanner**, you inspire me everyday with your passion for science and understanding. Life would not have been the same had I not taken Neurobiology with you. You make the most daunting things in science seem like simple jigsaw puzzles. I am such a fan. Thank you.

To **MHC & FC Dance-** Rose & Charles Flachs, Jim Coleman, Terese Freedman, Peter Jones, Paul Matteson, Allison Smartt, Gwendolyn Niven, Jean Baxter, Candice Salyers and Brenda Cortina. I had no idea an academic department could be filled with the same love, acceptance, warmth and pleasure that is only comparable to warm chocolate ramekins on a cold day. Thank you for being my family in art, learning and life.

To **Amy Camp,** thank you for exposing me to the wonderful world of proteins. Learning about them was like falling in love. I'm almost quite certain that you are Superwoman. Thank you for all your support and help.

To **All my Homeslices-** Paul Blackmar, Talia Zhang, Oshani Kulatunga, Dayita Nereyeth, Margaret Wiss, Megha Kymal, Celine Barreau, Lauren Altomare, Lindsey Spratt, Audrey Hildebrandt, Gautam Nima, Ishan Sharma, Ismat Imaan, Nakul Syal, Neha Sharma, Johanna Kneifel, Keya Chilka, Sanjana Gopinath and Priyam Turkhia. For every moment that I've needed you and you've always been there for me- Thank you.

To **My mom, dad and brother-** you've always taught me to be my best, never settle, and chase my dreams to the very end. Well, here I am and I can't thank you enough. I miss you all and love you very much.

To **Lynk UAF**, thank you for filling my stomach and fuelling my mind.

To ***The Harap Fund,*** for supporting me in my passions and endeavors- thank you.

To every science class I took, every success, every failure, all the strange conversations I have had at Mount Holyoke and every person who has helped me in any way. They say you only stand tall with solid ground beneath your feet. It would have never been the same without you.

To **Coffee-** I guess this entire thing would not have been possible without you. Thank you for being my friend, aide and confidante on long nights.

I dedicate this work to my grandparents, the ever loving and indulgent *Tatha, Patie, Bhapi* and *Maa*. Especially *Patie*- who is currently very, very ill. I hope I've learnt to be the scientist that you guys will always be proud of. I can't believe this is the end of a consuming process has helped me understand what I want to dedicate my life to. But then again this is not the end, it never will be.

It is always the beginning.

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INTRODUCTION

Autism Spectrum Disorders and the Social Brain

Autism Spectrum Disorder (ASD) is characterized along a spectrum of core symptoms comprising of severe and pervasive deficits in social communication and interaction, restricted, repetitive patterns of overt behavior, interests and activities (Woolfenden et al, 2011; DSM 5, American Psychiatric Association, 2013). Recent epidemiological studies have shown the steadily increasing prevalence of this condition between the 1990s to present (Matson et al., 2011); with 2.47 in 1990 (Ritvo et al., 1989) to 11 in 2002 (Croen et al., 2002), to between 60 and 70 per 10,000 children who were affected by this condition in 2009 (Fombonne et al, 2009) in the United States (Fombonne, 2005). Estimates for America from the Centers for Disease Control (CDC) showed a prevalence of 1 in 60 children affected by this condition in America (Center for Disease Control, 2012). A single global prevalence of this condition remains unknown (Elsabbagh et al., 2012), as the disorder presents with geographic localizations i.e. prevalence rates vary significantly between countries (Fombonne, 2005) and also amid urban and rural areas (Yeargin-Allsopp et al., 2003). Autism is unequally prevalent between the sexes, with males being about four times more likely to develop autism in comparison to females, and these rates have remained relatively stable over time (Fombonne, 2005). Interestingly however, the developmental profiles for communication, cognitive and motor deficits follow similar

trajectories between the sexes (Hartley & Sikora, 2009). It can be speculated that due to the presence of a global trend, there are likely similar factors that determine a diagnosis of ASD that are not entirely dependent on geography or socio- cultural factors, suggesting the relevance of factors like genetics and global environment change. In more recent years, increased awareness about this condition coupled with higher rates of reported and recorded cases are also potentially reflected in the increasing rates (Szpir, 2006).

There is currently no singular established etiology explaining the symptoms underlying this condition, but substantial evidence suggests both genetic and environmental factors in interaction, and in combination with multiple risk factors that cause the changes in brain development characteristic of ASD (Levy et al, 2009). The manifestation of the symptoms of ASD occurs along a spectrum i.e. they occur as a combination of the main symptoms that are specific to each individual (Schroeder et al, 2010). The autism "spectrum" is usually inclusive of Asperger's Syndrome, Pervasive Developmental Disorders (PDDs) and Autism, thus encompassing individuals who range from high functioning with no cognitive impairment to low functioning, with significant intellectual disabilities (Lord et al., 2000). The clinical definition of the spectrum differs according to the criteria used to diagnose it. In the USA, the Diagnostic and Statistical Manual (DSM) is used with frequent revisions to the definition of this condition. A confirmatory factor analysis study conducted by Mandy and colleagues (2012) showed that DSM-IV criteria inadequately described ASD,

while DSM-V criteria had greater construct validity as they describe the symptomatic experience as a dyad of social communication deficits and restricted, repetitive behaviors, including language, while sensory abnormalities were considered separately. The DSM-V criteria differentiate Autism from Social Communication disorders on the basis of severity creating a spectrum of behavioral deficits (McPartland et al., 2012). Social behavior disorders are incredibly variable in their nature; they range from disorders of excessive sociability but low cognitive function like William's syndrome (Mervis et al., 2000), to autism that is described by social behavior deficits, where the cognitive domain could be determined by mental retardation, visuo-perceptual and communication skills, or conversely, no impairments to cognition at all (Joseph et al., 2002). Similarly, Asperger's Syndrome is characterized by social behavior deficits, but little to no cognitive impairment (Schultz et al., 2000). Almost all such neurodevelopmental disorders that have social behavior deficits as a primary characteristic have genetics linked to synapse function implicated as a risk factor, though genetics alone is not enough to explain these conditions.

The symptomatic experience can be explained through the relationship between social motivation, attention, experience in a social setting and the resultant neuro-specialization that allows the developing brain to learn social behavioral and communication norms. Social behavior deficits can be speculatively correlated with the attention that is attributed to social stimuli (Grelotti et al, 2001). This further suggests that firstly, the symptoms of ASD

determine each other cyclically, and secondly; deficits occurring at any point along the cycle could both positively reinforce and create a wide array of symptoms (*Figure 1*). This co-dependent nature of development also points to the importance of neurospecialization of the brain for social interactions. Neurospecialization can be understood as the ability of the brain to adapt in favor of certain behaviors through learning and plasticity. This aspect of the reinforcement of social behavior is susceptible to the effect of both genetic and environmental factors that could influence synapse function, and resultantly, plasticity. Differences in the ability to learn and modify synapses can result in social behavioral deficits like those seen in ASD.

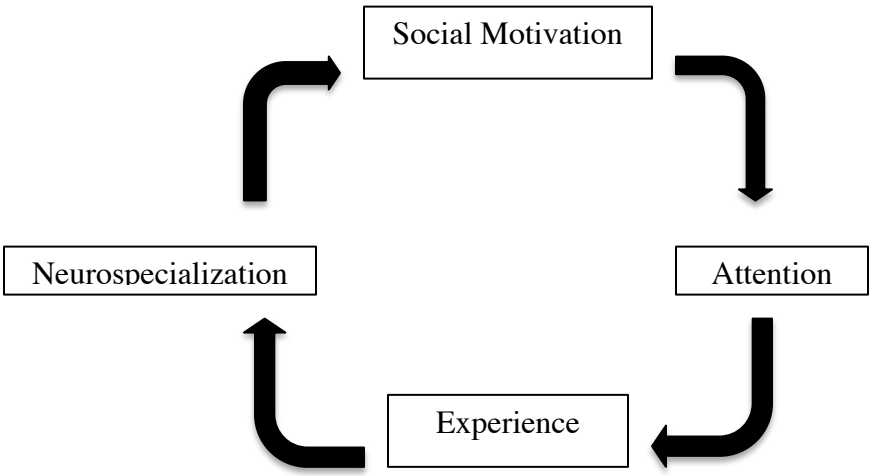

Figure 1- Cyclic Determination of Social Behavior during Neurodevelopment
Flow chart exhibiting how the elements of social behavior like motivation, attention and experience cyclically determine neurospecialization through development. Conversely, typical development of neurospecialization also mitigates the ability to preferentially engage in social behavior in later life. Adapted from Dawson et al., 2005.

This understanding of these disorders is contingent on the social brain hypothesis that attributes the disproportionately large brains in primates versus other vertebrates to increases in cortical areas to accommodate their complex social systems. This hypothesis further suggests that the human brain, descendant from primates, has evolutionarily evolved to favor social interaction for the maintenance of its larger cortical volume and function (in comparison to primates). Interestingly, there has been evidence in support of the existence of an unusual type of projection neuron in the anterior cingulate cortex of humans and larger primates alone, a neural area known to regulate autonomic and central cognitive processes (Nimchinksy et al., 1999) that are implicated in social processes. Additionally, the most significant factor that correlates to the difference between human and nonhuman primates is the amount of prefrontal cortical white matter (Schoenemann et al., 2005). These findings in conjunction suggest selective neural specialization through evolution in favor of more complex social structures and relationships, explaining why reward attributed to social interaction might have evolutionary, psychological and physiological benefits.

Alternatively, evolutionary selection in favor of the social brain in humans indicates higher perceived reward and resultant increased neuro-specialization from social interactions than nonhuman primates, and consequently primates more than other mammals. In the context of autism spectrum disorders, studies have implicated reduced social motivation resulting in the specific social

behavioral deficits. The DSM-IV (American Psychological Association, 1994) included lack of social motivation as a diagnostic criteria based on clinical observation, suggesting that individuals with autism have "a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people" and "lack of social or emotional reciprocity" (Wilson et al., 2013; DSM-IV, American Psychiatric Association, 1994). Observation and clinical studies have also shown that children with autism are more likely to find reduced reward in activities involving joint attention (Kasari et al., 1990), despite joint attention being implicated as potential behavioral therapy for autistic disorder (Whalen et al., 2006). Autistic individuals also lack the ability to seek and maintain social behavior with others (Chevellier et al, 2012). This social motivation hypothesis explains that reduced social motivation results in lowered attention to faces and all other social stimuli like the human voice, hand gestures and body language in children with ASD, which persist through adulthood (Dawson et al., 2002). That is, with reductions or absences of the rewarding value of social stimuli, children at a young age do not attend to social stimuli, failing to gain the necessary social experiences needed to build social skill and neurospecialization to process and navigate social environments. Dawson and colleagues (2005) also questioned the nature of the reward value of social stimuli, implicating from previous studies that behavioral deficits could result from anomalies in the reward system itself or neural networks pertaining to the positive perception of reward.

A "theory of mind" component is also present in the perception of social

stimuli that could be missing in individuals with perceptual deficits. For example, children with ASD might not be able to correlate "I smile when I am happy" with "he is smiling so he must be happy" he/she observes in another child due to lack of attention or perception (Williams et al., 2001). Impairments like this could explain why such a child perceives lower social reward, and hence is less motivated to do the same. The perception of reward, or the attachment of some valence to it is directly related to emotional processing; consequently, studies have found that representations of "reward value" within the orbitofrontal cortex depend largely on the strength of the signal from the basolateral amygdala, a region known to play a role in emotional processing (Schoenbaum et al., 2003). It can be speculated that since core cognitive processes like attention and perception strongly dictate social reward and motivation, there must be a subset of these larger neural processes that are further specialized to encode, remember and reproduce social stimuli. Furthermore, dopaminergic projections to the striatum and frontal cortex have been shown to affect reward and approach behavior (Schultz, 1998; Schultz et al., 2000), suggesting a reinforcement mechanism for social stimuli that are processed appropriately. Such evidence highlights pathways through which development in autism is different. Firstly, one theory suggests that individuals with ASD are not motivated to engage in social interaction. However, this notion is not entirely supported by the social brain hypothesis because if individuals with autism perceived less reward, there would be sub-threshold signaling to cortical areas and reductions in these neural pathways.

Alternatively, if there are differences in how such individuals process social stimuli i.e. they do not adequately specialize in order to perceive the entire "reward value" of a social interaction, then social stimuli are not positively reinforced at an early age, resultantly limiting future drive to engage in social interactions throughout development.

Social cognition can be understood as the individual's ability to engage in facial and emotional processing, as well as social pattern recognition. Typically, general face processing develops within the first few months of life, with heightened sensitivity to such stimuli developing later in adolescence (Batty & Taylor, 2006). Nelson and colleagues (2001) have shown that the development of facial processing might be experience dependent, suggesting that adequate exposure to faces during infancy without the presence of any attention deficits would specialize certain cortical and sub- cortical areas toward facial processing. Functional connectivity studies on neurodevelopment in the autistic brain have shown that there might be disordered connectivity that does not process the difference between social and non- social stimuli (Rippon et al., 2007). This could create cognitive and processing deficits, that are prevalent in how both social and non- social stimuli are encoded. Clinical diagnoses for broader Autism spectrum disorder phenotypes support this discrepancy; patients are often observed with mild, moderate or severe cognitive impairment in addition to social behavior deficits.

Facial processing itself can be understood as the ability to perceive faces as distinct stimuli and categorize them (Jolicouer et al, 1984), though there is no integral difference between faces and objects as stimuli per se (Tanaka & Taylor, 1991; 2001). Prior experience with stimuli, especially faces allows for the development and use of configural perceptual processing that occurs at a level subordinate to entry- level judgment i.e. facial recognition is quicker than object recognition (Yin, 1969; Valentine, 1988). In studies with autistic children, a higher perceptual inversion effect for faces, or the time taken to recognize a face in comparison to typically developing individuals was suggested (Dawson et al, 1998; Volkmar et al 1986) implying the low social salience and importance of facial stimuli in individuals with ASD, amongst other types of stimuli in their cognitive processes. There are three main hypotheses that explain the perceptual-cognitive bases of facial processing impairments in autism. These suggest that recognition deficits can be explained by either a fundamental inability to engage in perceptual binding, or a higher order deficit that prevents the extraction of perceptually relevant information from facial stimuli, or physiological dysfunction of the area responsible for facial processing i.e. the fusiform gyrus. Additionally defective function within the fusiform gyrus could impact larger social brain networks, creating secondary deficits in joint attention, interpretation of emotional expression and perception of other social stimuli (Dawson et al., 2005). Neural substrates of facial processing deficits have been implicated in the fusiform gyrus located in the ventral temporal lobe in patients suffering from

prosopagnosia (Damasio et al, 1982). A functional imaging study analyzing whole brain connectivity in the identification of social versus non-social stimuli found that greater clinical social impairment was associated with reduced Fusiform Face Area (FFA) to amygdala connectivity and increased FFA to right inferior frontal connectivity (Kleinhans et al., 2008). This significant relationship between abnormal functional connectivity and clinical severity in ASD suggests deficits to neuro-specialization, especially in cognitive domains involving facial processing, information encoding and potentially, retrieval.

The capacity to recognize patterns of social behavior and reciprocate them is strongly linked to social recognition ability, which in turn also inform the degree to which an individual can use context to determine the importance of social stimuli (Frith & Happe, 1994; 1996; 2006). Frith and colleagues (2006) suggest that the characteristic perceptual, cognitive, behavioral and social deficits typical to ASD cannot be determined by localization to any brain regions, instead the deficit may lie in cortical and sub cortical areas that play an important role in sensory integration at a semantic and perceptual level. Recent studies have shown that children from 2 years on with autism show a preference for geometric shapes over faces (Pierce et al, 2011). Though there is evidence to suggest that visual processing strategies develop atypically in individuals with autism using visual search tasks involving target and distractor stimuli (Kaldy et al, 2011), the nature of the difference in this evolving trajectory of social pattern recognition and social cognition is not understood. The ventral tegmental area has also been implicated

as an area where oxytocin regulates the social salience of stimuli that could play a potentially important role in triggering motivation in response to anticipated social reward or punishment (Groppe et al, 2012). There are confounds to these results because of the number of localized brain areas that are impacted by oxytocin signaling, along with the impact on the psychobiological processes of stress reduction and assignment of salience to stimuli (Insel T, 2003; Modi & Young, 2012).

Another important factor that affects social cognition is the emotional processing ability of the individual that involves the ascription of emotional, or "reward value" to social stimuli that impacts recall and repetition of these observed patterns and behaviors. Interestingly, there is evidence that suggests a facial affect recognition deficit in ASD (Ashwin et al, 2006). In ERP studies involving typically developing (TD) and autistic children, TD participants were able to distinguish between familiar and unfamiliar faces as well as objects while autistic participants were able to distinguish between familiar and unfamiliar objects, but not faces (Dawson et al., 2002). This suggests that autism like deficits do not allow such people to differentiate between social and non-social stimuli, a deficit that is cognitive in nature. Furthermore, other electrophysiological studies have found that individuals with high- functioning autism had heightened brain activity in response to happy faces, but did not mirror this effect in response to sad faces, which are crucial in communicating social error or empathy messages (Barrie, 2012). Hobson (2006) also showed that autistic adults were worse than

typically developed adults at labeling emotional states in relation to faces as well as whole- body biological movements. Visual scan path studies of autistic and typically developing individuals further showed that autistic adults spent more time focused on non- core areas of the face (everything except the eyes, nose and mouth) (Pelphrey et al., 2002). These studies highlight processing strategies required for successful social cognition (like focal processing areas), indicating that individuals with autism do not develop these cognitive strategies, and hence are less likely to engage in appropriate social behavior.

These differences in cognition and processing are also prevalent at a physiological level. A PET based regional cerebral flood flow (rCBF) study measuring the response of autistic and typically developing individuals to facial stimuli in conjunction with prosodic information showed that autistic individuals had lower rCBF in the interior frontal and fusiform face areas. Conversely, they had higher rCBF levels at the right anterior temporal lobe, the anterior cingulate and the thalamus (Hall et al., 2014). This showed that individuals with autism showed lower activity in fusiform areas related to recognition while showing heightened activity in limbic and cortical areas related to emotional salience, accompanied by a lack of integration with emotional processing that does not allow the association of "social reward" to incoming stimuli.

Functional imaging studies in typically developing individuals have identified two areas linked to social cognition in the lateral occipito-temporo-parietal cortex, that have implications for emotional processing abilities. These

are the posterior superior temporal sulcus region for understanding action (Saxe et al., 2004) and the temporo-parietal junction area for representing mental states (Saxe & Kanwisher, 2003). The variability in neural correlates implicated in social recognition can be attributed to the heterogeneity prevalent in autism, along with differences in testing methodology (Kennedy & Adolphs, 2012). Furthermore, it can be speculated that since significant deficits in social cognition and recognition occur despite substantial evidence to support correlated affect perception deficits, emotions do not play a salient role in facial recognition in autistic individuals. This further suggests the increased likelihood that social behavior deficits are likely linked to developmental differences in cognition. Hall and colleagues (2014) also suggested that autistic individuals utilize a different strategy for facial emotion recognition, one involving attention, categorization and the referencing of perceptual knowledge, which could be indicative of the mediating role of memory.

An eye- tracking study on TD and autistic children using self, familiar, unfamiliar and facial images have suggested that attention to the eye area of unfamiliar and self faces was correlated with socio- communicative ability (Gillespie- Smith et al., 2014). Additionally, an fMRI investigation on visual coding of faces and working memory showed that individuals with autism had lowered inferior left prefrontal area and right posterior temporal area activation compared to controls. These areas are associated with verbal processing and working memory maintenance and theory of mind processing respectively

(Koshino et al., 2008). Speculatively, this reduced recruitment of frontal memory related areas could account for facial recognition differences seen in autism. Though very little research has been done on the implications of social memory on facial recognition, a study by Hauck and colleagues (1998) showed that autistic children were impaired relative to TD children on the social face memory task only, and this task amongst others had the highest and most consistent correlation with measures of social development and adaptive skills. Another study determined that autistic children were less able to distinguish unfamiliar faces than houses from familiar ones in comparison to their age- matched controls, while there were no reported differences in fixation between groups (Boucher & Lewis, 1992). These results suggest that deficits might not be due to impaired attention or discrimination ability; social memory might be playing an important role in the salience of familiar and unfamiliar stimuli.

The interface of these various factors inform other higher order functions that are involved in social interactions, like social recognition and cognition. It has also been shown that the global occurrence of social motivation in all children with an autism diagnosis correlates with deficits in social cognition by consequence, not correlation (Baron- Cohen, 1955). There are many physiological markers that are also implicated in individuals with low social motivation i.e. specific hormones that regulate sociability (oxytocin) and stress (cortisol) which influence social interactions in early childhood and formative years (Honey et al, 2007). For example, a child with higher cortisol in social settings is less likely to

engage in age- appropriate play that is critical for social learning through attention and observation. Research in an animal model of social behavior deficits has found that CD38, a transmembrane protein regulates sociability in mice by altering oxytocin secretion in the brain. Additionally, CD38 knockout mice demonstrated social amnesia due to diminished oxytocin function (Higashida et al., 2012), further supporting the idea of the existence of social memory as a discrete neural process dependent on oxytocin signaling as a biomarker in both the brain and plasma. Also, a study on autistic versus control individuals found that people with autism had lower levels of plasma cortisol but higher levels of ACTH (adrenocorticotropic hormone), suggesting an imbalance in the HPA (hypothalamic-pituatary-adrenal) axis (Curin et al., 2003). Furthermore, Curin and colleagues (2008) also show that autistic individuals had a slower cortisol awakening response after ACTH stimulation than controls, further validating the HPA dysfunction hypothesis. It can be theorized that HPA dysfunction leading to higher stress in novel social recognition and memory situations could be an indicative biomarker for autistic like behavioral dysfunction in an individual.

The social motivation hypothesis provides a model that helps understand the interaction of facial processing, cognition and memory in lieu of social stimuli, suggesting that aberrant neural responses to human faces in autism can be explained by atypical social development, and consequently reduced exposure to faces. This model is based on the assumption that in a typically developing individual, paying attention to social stimuli like faces would be rewarding and

positively reinforced. Atypical social development could arise in situations where individuals do not derive the same amount of reward out of social interaction, hence affecting the way it is reinforced. This discrepancy can either occur at the stage of import and information processing of social stimuli (Dawson, Web & McPartland, 2005), or as a result altered connectivity between different brain regions (Minshew & Williams, 2007). Social brain theories have argued as to the relevance of social stimuli in brain development i.e. does brain development specialize to better encode social stimuli or is the nature of social stimuli inherently different from other stimuli? Furthermore, studies have shown that electrophysiological brain activity in response to faces can serve as a significant bio- behavioral risk marker for ASD, as temporal anomalies in brain regions connected with facial perception have been observed in children with autism (Dawson, Wed & Wijsman et al, 2005) and at- risk infants (McCleery et al, 2009).

Social recognition can be understood as a further breakdown of the information import based theory of the social brain- do social behavior deficits occur due to an inability to recognize the inherent difference between social vs. non- social stimuli? Does this difference impact the reward and reinforcement structure of these stimuli, and consequently the neural specialization? A study conducted by Rochat and colleagues (2013) showed that children with ASD are impaired relative to TD children in understanding why an agent is performing a certain action i.e. the intention behind the action. Subsequent work with mirror neurons (Rizolatti et al., 1996; Cattaneo et al., 2007) and “vitality forms” (Stern et

al., 1995; 2010) of social stimuli i.e. action dynamics that provide insight into the cognitive/ emotional state of the performer point toward the need for adequate recognition and processing faculties that can encode the salience of this information. Social recognition, as an offshoot of social cognition, can be speculatively defined as the ability to recognize and encode social stimuli by optimizing its social reward structure in neural specialization toward adaptive social development.

Risk factors such as genetic predisposition, environmental pathogen exposure or a combination of both are implicated in neurodevelopmental disorders like ASD that are characterized by social behavior deficits in motivation, cognition and memory. Furthermore, it is important to categorize how these risk factors might specifically impact the development of processing, cognition, memory and motivation to engage in social interactions by changing brain structure, function and connectivity during development.

Maternal Immune Activation

The symptoms of ASD have been explained by two major causative factors, namely genetics or environmental insults. One such environment-based postulate is the maternal immune activation (MIA) hypothesis that describes the relationship between insults to maternal immunity during pregnancy and how that affects the development of the offspring i*n- utero* (Brown et al., 2012). Epidemiological studies have shown that mothers exposed to viral infections, for

example, Influenza (Mednick et al., 1994) or high- grade fevers, during their second or third trimester are highly correlated with the birth of offspring that are born with behavioral and social deficits reminiscent of autistic symptoms (Zerbo et al., 2013). Though there is variability present in whether this effect is more prominent when the environmental insult is experienced in the second versus third trimester, the presence of a strong correlation nonetheless suggests the salience of this hypothesis. Replicated studies have shown that this effect sustains across geographical boundaries implying that the insult must be globally prevalent, making a strong case for investigating the impact of common environmental insults like fever (Atladottir et al., 2010; Libbey et al., 2005).

MIA is described as the heightened immune response of the mother during pregnancy in reaction to environmental insults. Despite being the normal mechanism of defense against environmental pathogens, the coincidence of this elevated immune response has been shown to chemically alter neurodevelopment (Zuckerman & Weiner, 2005). These developmental differences have been implicated as increased risk factors for behavioral disorders like Autism and Schizophrenia (Patterson, 2011), indicating that it might be some aspect of the immune response and not the pathogen itself that is causative of these conditions.

A variety of pathogens are related to altered neurodevelopment via interaction with the maternal immune system. In both schizophrenia and autism, investigations into prenatal exposure have shown that viral transfection might be mediating developmental differences, since in utero viral exposure has been more

generally implicated in both gross and subtle congenital anomalies of the central nervous system (Brown & Penner, 2007). Furthermore, this exposure is indicative of adult immune dysregulation, a condition that grows out of neurodevelopemental disorders. There is evidence suggesting the altered behavior of immune- related genes in the schizophrenic brain (Arion et al., 2007), and similarly, astrocytic and microglial activation and cytokine up-regulation in the autism brain (Vargas et al., 2005). This resultant altered, yet subclinical immune state has been found to be present in childhood and persist into adulthood, resulting in consistently heightened cytokine and chemokine function that could be harming instead of helping brain development. Prenatal exposure to rubella has been linked with a larger incidence of both autism and schizophrenia in offspring, maternal bacterial infection with pathogens like the cytomegalovirus has also been shown to greatly increase the risk for autism in mouse models (Moy & Nadler, 2008). Though the direct causative mechanism is unknown, such exposure has been shown to not only alter the immune state of the fetal brain and central nervous system, but also affect the formation of the offspring's entire peripheral nervous system by altering developing hematopoietic stem cells due to viral pathology present in the developing placenta (Anderson et al., 2007).

Epidemiological data from longitudinal birth cohort studies in humans in Europe and America between as early as 1988 (Mednick et al.) and last reported in 2003 (Limosin et al.) have shown that exposure between the end of the second and the beginning of the third trimester to influenza can be considered as a

potential risk factor. A study by Brown and colleagues (2006) showed a significantly higher incidence of schizophrenia and autism when the immune insult was experienced in the second trimester and conversely, early in the third trimester. Difficulties in the replication of these cohort studies including anonymity in the definition of the diagnosis of an Influenza infection have allowed for this model to be broadened to many other environmental pathogens (Brown & Derkits, 2009). It is evident from these findings that the time of immune response in the mother and the related developmental stage of the foetus determine the nature of the deficits. Between trimesters, these developmental impairments subtly differ but also show altered brain pathology in similar regions implicating that in utero exposure could be responsible for neurodevelopmental disorders that have both behavioral and physiological differences.

Animal models have been extensively used to study the downstream effects of maternal infection on social motivation and cognition, deficits to which have been found in neurodevelopmental conditions. Models of mid- gestation respiratory infection via Influenza have shown histological differences in both the hippocampus and cortex, areas closely linked with cognition and memory capabilities. Offspring prenatally exposed to Influenza also show abnormalities in social behavior, pre-pulse inhibition, open field exploration and novel object recognition, suggesting behavioral abnormalities in exploration, anxiety and sensory- motor integration. Behavioral assays like social approach, reciprocal social interaction, socially conditioned place preference and social recognition

that specifically address social behavioral deficits have been used genetic mutant mouse models to characterize behavioral deficits specific to autism (Crawley, 2007). However, these assays have not been applies to study differences between species typical and prenatally exposed offspring. These create reliable translational models especially for disorders like autism, where arguably the most clinically significant symptom is social behavior deficits.

Since there has not been sufficient replication and revalidation of Influenza mediated neurodevelopmental differences, alternative animal models that hope to understand the immune mechanism more than create correlational specificity with a particular viral pathogen have been employed. These mimic an anti-viral inflammatory immune response in the mother by transfection with synthetic double stranded RNA in the absence of a viral pathogen. Polyinocytic-polycytidilic acid (Poly I: C), one such model, uses dsRNA that acts through the Toll- like receptor-3 pathway, and is sufficient to cause all behavioral and physiologically abnormalities associated with MIA offspring (Patterson, 2009). This model has shown deficits in working memory, pre- pulse inhibition, social interaction and novel object exploration, implying developmental deficits in cognition, memory and social behavior. Additionally, this model has also found altered $GABA_A$ receptor immunoreactivity, dopamine hyperfunction (specifically in schizophrenia), reduced hippocampal myelination (Manikodan et al., 2008) and NMDA receptor expression along with reduced reelin and parvalbumin positive cells and D1/ D2 receptors in the prefrontal cortex (Meyer et al., 2008). The

neurological localization of these physiological deficits indicates an altered signaling and chemical balance within the brain, and speculatively incomplete development of the structures themselves.

Another method of MIA induction is via the Toll- like receptor 4 (TLR-4) mediated by bacterial infection. Intraperitoneal injection (single or double doses) with lipopolysaccharide, or LPS mid- gestation has been shown to cause similar deficits in social interaction, processing, learning, working memory and increased anxiety. These deficits are similar to those induced by Poly I:C, further strengthening the hypothesis that perhaps the elevated immune response itself is more important than the specific pathogen responsible. Interestingly, both these models of MIA induction lead to cytokine-mediated neuroinflammation characterized by astrogliosis and heightened microglial immunostaining (Jonakait, 2007) found in both schizophrenia and autism that sustain into adulthood. Increased numbers of both astrocytes and microglia normally occur in the presence of an immune insult, and have implications for energy metabolism, regulation of blood flow, ionic/ transmitter homeostasis and synaptic function and remodeling. Poly I: C has also been used in murine models of schizophrenia, showing significantly impaired sensorimotor gating ability and reduced amount of prefrontal dopamine D1 receptors (Meyer et al., 2008). Behavioral assessments conducted using the Poly I: C model found deficits in a variety of tasks, including marble burying and social approach, implying deficits in social motivation and increased anxiety and/ or repetitive behaviors (Schwartzer et al., 2013; Onore et

al., 2014). Changes in brain physiology of MIA offspring mice have been implicated (Garay et al., 2009), also accompanied with changes in behavior that are comparable to the deficits characteristic of ASD (Malkova et al., 2012). Murine models using Poly I: C have found working memory deficits (at 5mg/kg) (Ozawa et al., 2006), increased locomotion and open field activity, with altered dopamine sensitivity when administered at embryonic day 12 (20mg/kg) prevalent in striatal, hippocampal and cortical regions (Smith et al., 2008). Specifically, from the nature of Poly I: C mediated behavioral abnormalities and the resultant functional differences present in areas of the brain strongly associated with memory, cognition and processing, it can be speculatively inferred that developmental deficits in these regions could also be potentially causative of social behavior deficits in memory, recognition and processing, all of which are symptoms associated with autism.

The mediating causes of this model are currently not completely understood i.e. how do immunity-linked changes in homeostasis of the mother affect or alter the development of the offspring (Li et al., 2009)? Recent research has supported the role of infection related elevated cytokines and their passage to the offspring through the bloodstream and placenta of the mother or via symbiotic release of similar cytokines within the offspring itself to establish an equilibrated environment (Brown et al., 2013; Ashwood et al., 2011). Studies with LPS injections into the uterine horn in mouse models have shown elevated CNS pro-inflammatory cytokines like IL-6 and TNF-α in both the placenta and the

amniotic fluid (Jonakait, 2007), mirroring the physiology of an immune response. Additionally, the same cytokines have been found in fetal brains (Patterson et al., 2006), suggesting some interaction between the mother and the fetus specifically affects the developing immune system by positively reinforcing a state of generalized inflammation via cytokines and their signaling pathways in the neonatal brain.

Studies have shown generalized inflammation in the brain and other organs in autistic patients both post mortem, as well as in individuals ranging from 5 to 44 years old (Pardo et al., 2005). This variation strongly suggests that altered neural development as a result of maternal immune activation is established early, probably in the uterine environment, and is a permanent change (Patterson, 2011). Though genetic based etiology first evolved out of twin- studies in the 1970s, more recent research using sequence analyses have shown highly penetrant copy number variants (CNVs) and single nucleotide variants (SNVs) in ASD patients, further supporting a genetic etiology to autism along with explaining the inherent heterogeneity of the spectrum (Jiang et al., 2014). However, though genetics may confer an increased risk that can be compounded by an immune insult, MIA can also affect gene transcription and translation in the developing brain. Poly: IC has also been used to study gene expression within the fetal brain as a result of maternal infection. Garbett and colleagues (2012) found a strong up-regulation of the crystalline gene family, which was correlated with the severity of the induced maternal immune response as assessed through placental

weight. They also suggested that changes in gene expression could be a neuro-protective adaptation while the developing brain is in a constant state of "stress" that affects neural differentiation and axonal growth. Though genetic based etiology first evolved out of twin- studies in the1970s, more recent research using sequence analyses have shown highly penetrant copy number variants (CNVs) and single nucleotide variants (SNVs) in ASD patients, further supporting a genetic etiology to autism along with explaining the inherent heterogeneity of the spectrum (Jiang et al., 2014). Though many genes have been implicated in the symptoms that characterize ASD like GABRB3 (Chen et al., 2013), SHANK3 (Wang et al., 2014) and other loci like 7q11 (Nijmeijer et al., 2014), there is strong evidence for a variety of gene- environment interactions that determines the manifestation of the autism condition (Meek et al., 2013). Additionally, genes coding for pro- inflammatory cytokines are upregulated in the autistic brain, along with over expression of IL-6 mRNA in the hippocampus. Consequently, Samuelsson et al. (2006) showed that exposure to IL-6 alone was sufficient to induce memory deficits in rats that persisted into adulthood. This implies that not only might IL-6 be one cytokine responsible for the developmental differences observed in autism amongst a matrix of others, but also that immune- linked genes are modulated by environmental insults causing behavioral differences like those in autism. A similar argument was employed in a study that examined how administration of IL-6, a pro- inflammatory cytokine known to be a physiological mediator of MIA- linked neurodevelopmental changes, to C57 mice was

sufficient to induce latent and pre-pulse inhibition differences that are reminiscent of schizophrenia. The authors found that Poly I: C offspring showed no chamber preference in comparison to controls, and this effect was reversed with the co-administration of an IL-6 specific antibody (Smith et al., 2007). This study also suggests MIA- mediated alterations in gene expression in the fetal brain by identifying alterations in prefrontal cortex mRNA as a result of IL-6 exposure, where this area shows functional, molecular and microanatomical alterations in cognitive disorders (Lewis & Levitt, 2002).

Mouse Models

Limited opportunity for controlled experiments and long life spans allow these mechanisms to be more effectively modeled in animals as looking at fetal neurodevelopment and social behavior post birth in comparison to species typical behavior in a mouse model provides insight into the causative extent of the MIA hypothesis. Strong genetic conservation, along with shorter life spans and fewer ethical restrictions in conducting controlled experimental research allow for the construction of animal models that provide reasonably accurate causal information about how mechanisms like maternal immune activation might mediate deficits in neurodevelopment. The C57 mouse in particular makes for an excellent target model organism specifically for the study of how maternal health during pregnancy affects offspring neurodevelopment as they have a lifespan of 2 years on average, with frequently occurring estrous cycles, a short gestation cycle

(19 days on average) that allows for ease in conducting timed and monitored pregnancies. Additionally, they have an average litter size of between 8-12 pups that allows this mechanism to be validated in a large cohort.

The relatively short life span of this strain of mouse allows for the study of the development of social cognition and memory from infancy into adulthood. Additionally, histological and/or behavioral studies can be conducted at any time through the life span of the animal, allowing for comparative analyses that provide insight into how cognition and memory specialize to cater to social stimuli, a processing strategy that might be impacted by MIA.

C57 mice, and mice in general, do not depend on their vision as the primary sensory modality used for information import. In contrast, they are olfactory dominant, suggesting that they would process and perceive novel stimuli via odors versus actually seeing them. In relation to social stimuli, these mice would initiate social interaction in response to a novel odor from a mouse they have never met before. Alternatively, if MIA does mediate social cognition and memory deficits, these species typical mice when exposed to an immune insult in utero should not be able to differentiate between novel and familiar mice as they would not be sufficiently neurospecialized to encode and remember social stimuli. This model helps to understand how MIA- mediated developmental differences does not allow individuals with neurodevelopmental disorders like autism to perceive and remember social stimuli resulting in lowered motivation to initiate and maintain novel social interaction. This model also considers how MIA-

mediated deficits in cognition and memory might develop differently from species typical development at different ages, namely juvenile and adulthood. This is highly translational as neurodevelopmental disorders like autism in particular are often diagnosed in early childhood, where the clinical symptoms sustain all through life. Creating a causative link between MIA and developmental differences in cognition and memory imply that these deficits should be present, sustain and/or increase from juvenile to adulthood, making the mouse a good model for neurodevelopmental disorders that are characterized by social behavior deficits.

To determine whether MIA specifically affects the ability to form a preference for one environment over the other, when neither have any social associations, studies have used drug conditioned place preference tasks in rats and mice. Richtand and colleagues (2011) validated this paradigm in a Poly I:C MIA model using Sprague- Dawley rats to determine its effects on drug, specifically amphetamine conditioned learning and memory behavior in interaction with post-natal developmental stress. They found an increased drug- associated chamber preference in comparison to baseline prevalent in both the Poly I:C and PBS groups. In their longitudinal analysis pre and post treatment, they also found that Poly I:C offspring exposed to postnatal stressors were more likely to longitudinally develop a drug- conditioned place preference, while Poly I:C offspring in general spent more time in the drug- conditioned environments than PBS offspring. These data suggest that Poly I:C mediated MIA does allow

offspring to make context specific associations, but could be affecting their cognition and memory specifically as they repeatedly spend more time in a conditioned chamber despite being familiar with it. Possible confounds to this interpretation include the interaction between Poly I:C and drug administration, though this study still demonstrates that Poly I:C offspring have the ability to make conditioned associations, implying that something might be different in the condition of social stimuli.

There are behavioral assays that specifically address the question of conditioned social preference, implying that rodents exhibiting species typical behavior should prefer to spend more time in a chamber associated with social interaction. A study conducted by Oskvig and colleagues in Sprague- Dawley rats showed a reduced social preference in offspring of dams exposed to LPS in comparison to controls, where LPS offspring spent significantly less time investigating the social chamber. The authors conducted this task in 5 trials at various ages through development, and found that LPS offspring spent less time in the social chamber in the first four trials, with no difference in the fifth. Analysis of their protocol (Walker et al., 2007) shows that their social chamber included a novel stimulus mouse while their familiar chamber included bedding from their own cage. Despite the presentation of treatment effects, the inherently different nature of stimuli (bedding compared to a novel mouse) could be a potential confound. Additionally, the authors used an olfaction dependent analysis to determine social preference that does have a cognitive domain, but did not

address how LPS induced MIA potentially affects the development of cognition. Their methodology also does not address questions of the development of social memory. Extrapolating these findings to autism specifically, it can be speculated that MIA induced neurodevelopmental deficits impacts cognition that affects the encoding of memory, or affects memory specific to social stimuli while leaving cognition intact still resulting in differences in social preference.

Social behavior has been studied using various paradigms that validate a certain aspect of social interaction, modeling deficits often seen in social behavior disorders like the inability to initiate novel social interactions and a lack of social motivation. The social approach task provides a method of investigating the nature of social behavioral deficits in mice by identifying their preference for a novel mouse versus novel object, implying that these mice would typically spend more time with the novel mouse than object as they find initiating and engaging in social interaction rewarding. Tests conducted with genetically engineered mouse lines relevant to autism have shown reduced interest in social contact, approach and proximity in mice with induced mutations of the FMR1 gene, polymorphisms in the SERT, or serotonin transporter gene and induced overexpression of the IgF-1, or Insulin-like growth factor-1 gene that postulate a model for autism- linked brain overgrowth (Moy et al., 2009). This task was used by Schwartzer and colleagues (2013) in conjunction with Poly I:C mediated MIA to determine differences in social approach behavior, showing that offspring of exposed dams had reduced social preference for the chamber with the novel mouse in

comparison to control offspring. Though this task addresses questions of whether or not these mice prefer social interaction to an asocial stimulus like an object, it does not address the question of social preference between two social stimuli, either known or novel is impacted by maternal immune activation. This has translational relevance to the experience of individuals with autism who might have developmental differences in social cognition and memory that do not allow them to successfully differentiate between novel and familiar social stimuli, affecting their ability to initiate novel social interaction.

An adaptation of this task has been implicated in studies addressing differences in social recognition, suggesting that social behavior deficits might be caused by either processing deficits, memory deficits or a combination of both that might be occurring either upstream or downstream of each other. Data from the social approach task is often interpreted as having implications for cognition and processing deficits, however it is hard to discern these differences as the nature of stimuli used (mouse versus an object) are inherently different, i.e. the object can have a social association but a novel mouse is a social stimuli in itself. This further evidences that social behavior differences could be mediated by cognitive deficits, and hence needs to be studied using a task that addresses this specifically. Additionally, MIA has also been shown to exacerbate social recognition deficits in heterozygous mice with two distinct mutations in the *Disc1* gene, supporting a model for the development of schizophrenia symptoms in adult mice. In contrast to social approach, in this study the test mouse was introduced to

a novel mouse for a ten minute session and then compared to a new novel mouse, assuming that these mice would be able to establish recognition memory of the first novel mouse within the 10 minute session. This leaves room for possible confounds due to memory differences that could be impacted by Poly I:C induced MIA. Results showed that offspring that had received Poly I:C and also had the Disc1/L100P mutation showed no preference between the first and second novel stimulus mouse (Lipina et al., 2013). A study conducted by Zhang and colleagues (2014) found that GAD67 knockout mice i.e. mice that are unable to synthesize GABA were more likely to spend time in the chamber with a mouse they had prior experience with, or the first stranger mouse. These studies demonstrate the use of a social recognition task to measure changes in social cognitive processes but do not address questions of how maternal infection alone might impact the same preference. Additionally, the social recognition task limits the nature of the deficit to being purely cognitive, assuming that if the MIA offspring do not make a preference for either mouse, they have developmental differences in how they process these social stimuli. However, it is not known whether MIA can specifically mediate short term or long-term memory deficits, or whether MIA mediated physiological developmental differences specifically affects how social stimuli are encoded and stored in memory. In contrast to the social approach task, using a novel mouse versus a littermate mouse instead of the novel object specifically addresses if MIA mediates any developmental differences in the perception of social stimuli. Secondly, in comparison to the social recognition

task, allowing the experimental mouse to display a preference between either a novel same-sex and same age mouse versus a littermate mouse that it has been co-housed with can suggest the length of social exposure required to construct a social memory, how it is maintained over time and lastly how MIA impacts this process. This study attempted to describe these differences by conducting this task at PND30, a juvenile age, and conducting the task with the exact same pairings of novel and littermate mice again at PND60 i.e. adult age to establish whether these social cognitive and memory differences are maintained with developmental age. Additionally, this tasks attempts to determine whether the formation of social memory that occurred at PND 30 is firstly different from controls, and secondly, whether it is maintained over time. MIA has shown negative effects on memory retention, suggesting that short-term exposure to the novel mouse could greatly confound experience- based social recognition (Patterson et al., 2011). Additionally, the inherent behavioral differences between Poly I: C and saline mice are not known, which must be taken into account. Long-term exposure and related stronger memory formation (Lynch, 2004), as would occur in the social relationship between two littermate mice reduces memory linked confounds when compared to a novel mouse. Additionally, to define whether these memory deficits are short-term, long- term or both, at PND 60, the social preference of experimental mice is tested between the original stranger mouse (when compared to the littermate) and a new novel mouse. This paradigm provides comprehensive information not only about how MIA might be affecting the development of

cognition and memory formation, but also the nature of the memory deficit and how these differences manifest at different developmental ages. This model also mimics the social experience of autism for human beings more realistically than comparing a short- term exposure to a novel mouse, better modeling why individuals with autism lack social motivation and related cognitive skills to initiate novel social interactions.

This study uses this altered social recognition paradigm that targets social cognition by determining the inherent social preference of C57 mice between littermate versus novel mice. Additionally, this task addresses the impact of maternal immune activation on the development and maintenance of social memory, and the nature of the memory deficit. It was hypothesized that pups of Poly I: C dams will show a preference for littermate mice compared to controls at both PND 30 and PND 60 by spending more time in interaction with the novel mouse. Additionally, I hypothesize a differential effect for direct versus peripheral social interactions, suggesting that Poly I: C pups will spend more time directly interacting with littermates, but will engage in peripheral interactions with the novel mouse, providing more qualitative information about the nature of the social interaction. Additionally, when briefly introduced to a completely novel mouse in comparison to the "acquainted" novel mouse, Poly I: C offspring are hypothesized to display preference for the newly introduced mouse, suggesting that MIA is also causative of memory and processing differences in conjunction in reference to social stimuli. This model will establish the relationship between

socio- cognitive deficit and memory, creating a comprehensive understanding of the nature of social behavior deficits mediated by maternal immune activation.

METHOD

Animals

C57Bl/6J (C57) mice were bred and kept at Mount Holyoke College, South Hadley at ambient room temperature on a 12-hour day/night cycle (7.30am-7.30pm) with food and water available at all times. All mice were group-housed in standard corncob bedding. All procedures were performed in approval and compliance with the regulations of Mount Holyoke College's Institutional Animal Care and Use Committee and the animal care guidelines for ethical treatment provided by the National Institutes of Health. Mice were bred in three separate cohorts; the first cohort was used to validate the altered social recognition paradigm, and the second and third cohorts were used to determine if MIA mediated any social recognition deficits.

Maternal Immune Activation (MIA)

Mice were mated with litter-mates overnight and females were checked daily for seminal plugs approximately 15 minutes after the end of their dark cycle. If a plug was found, the date was noted as gestational day 0.5 (G0.5) and the initial weight of the dam was recorded. Maternal Immune Activation (MIA) was induced at gestational day 12.5 (G12.5) i.e. 12 days after detection of the seminal plug by weighing the dams and injecting them intra-peritoneally with a single dose of either Poly I:C (20mg/kg) or saline solution. Each injection was administered at a volume of 4ml/kg with standard procedure for intra-peritoneal injections,

including initial verification of needle placement to insure no injury to surrounding internal organs and aspiration after insertion of the needle. Dams were then be returned to their cages containing standard enrichments, and left undisturbed until parturition. Pups were weaned away from the dam on postnatal day 21 (PND 21) and group housed in cages of between 2-4 mice with same sex littermates, depending on the size of the litter. Behavioral data was obtained from four separate cohorts. The first cohort was used to establish a baseline preference using the altered social recognition paradigm without any treatments in C57 mice. The second cohort contained n= 4 dams, (Poly I: C, n=2; Saline, n=2), with a total sample of n= 19 (Poly I: C, n= 9; Saline, n= 10). This cohort comprises of 11 males (Poly I: C, n=5; Saline, n=6) and 8 females (Poly I: C, n=4; Saline, n=4). The third cohort contained n= 4 dams (Poly I: C, n=1; Saline, n= 3), with an average on 8 pups with litter. This cohort had a sample of n= 30 (Poly I: C, n=7; Saline, n=24). There were a total of 17 males (Poly I: C, n=4; Saline, n=13) and 13 females (Poly I: C, n=3; Saline, n=10). The fourth cohort contained n=6 dams (Poly I: C, n= 3; Saline, n=3), with an average of 6 pups per litter (SD= 2). This cohort had a sample of n= 34 (Poly I: C, n= 20; Saline, n=14). There were a total of 14 males (Poly I: C, n=10; saline, n=4) and 20 females (Poly I: C, n= 10; saline, n=10).

Three- Chambered Social Recognition Paradigm

At PND 30, Poly IC and Saline offspring were assessed for social recognition using a modification of the social approach paradigm (Schwartzer et al., 2013) and social recognition paradigm (Lipina et al., 2013). Briefly, males and females from each litter, both Poly I: C and saline, were habituated for 10 minutes in a three- chambered behavioral box by placing the experimental mouse in the central chamber with the gates closed. The experimental mouse was then habituated to the entire apparatus by lifting the gates and allowing the mouse to freely explore the entire behavioral space for an additional 10 minutes. Following habituation, the mice were placed back in the central chamber and the gates closed. Inverted metal cups were placed in each of the side chambers; one containing a littermate mouse specific to the experimental mouse (the littermate mouse must have been housed in the same cage as the experimental mouse), and the other cup containing a novel mouse. In the first and second cohorts, the novel mouse of the same sex was chosen from a different litter, regardless of whether it was exposed to Poly I: C or saline, which may present a potential confound due to inherent behavioral differences between Poly I: C and saline mice. This was done in order to have all three mice (stranger, experimental and littermate) at approximately the same age to avoid aggression/ dominant behavior linked anxiety in the experimental mouse. This confound will be addressed in future cohorts as they are speculated to contain a larger sample size, making it probable to have novel mice from the same treatment group. Experimental mice comprised of offspring from both Poly I: C

and saline treated dams were given a 10- min test period to freely explore the entire apparatus after the gates were lifted. This session was video recorded and the behavioral videos later analyzed for chamber preference, interaction zone choice (whether direct or peripheral interaction with respect to the stranger or littermate chamber; *see Data Coding section below*), R (recognition) scores and locomotor activity (distance and velocity covered during 10 minute test period). Analyses were based on how much time the mouse spent in the chamber with either the littermate or novel mouse. All testing chambers were thoroughly cleaned with 70% Ethanol in between test sessions and scrubbed between different testing rounds. Experimental mice were sometimes used as stranger mice for a different experimental mouse, but they were always given a 24-hour or longer gap between each testing session.

At PND 60, Poly I: C and saline offspring were assessed for social recognition using the same protocol described above. The experimental pairings of stranger, littermate and experimental mice were kept exactly the same at PND 60 such that social recognition deficits, if found, could be studied longitudinally within the same subject. Additionally, a third test round was included within the same test session, where the social preference of the experimental mouse was determined based on a familiar novel mouse (the same mouse used when compared to littermate) in contrast to a brand new novel mouse, to further affirm the presence of a MIA- induced cognitive and memory deficit specific to social

stimuli. Data was collected using the same parameters mentioned above over a 10-minute test session.

Data for chamber preference, interaction zone choice and locomotor activity were analyzed by combining both PND 30 and 60 into a single model to firstly determine the nature of deficits, determine if behavioral trends in social recognition and memory are consistent between juvenile and adult animals and to further see whether these effects persist over time.

Data Coding

Behavioral data was coded using EthoVision software (Noldus) by personalizing the Arena Tracking settings. The experimental animal was measured for the time spent in each chamber i.e. the center, and the chambers with the stranger and littermate mice. Additionally, analysis zones were set up in concentric layers for each of the side chambers to understand how much time the mouse spent engaging with either mouse as opposed to time spent exploring the chamber. The rectangular chamber space was subdivided into two zones; a larger, centrally located outer square zone and an inner circular zone enclosing an additional area around the metal cup (Fig 2). Time spent within the square zone was analyzed as a peripheral social interaction, while time spent within the circular zone was scored as a direct social interaction. Times spent in each of these zones were considered exclusive of each other, and separate from time spent in the entire chamber. Additionally, an R score was calculated for each animal but subtracting the percent time in the littermate chamber from the percent time spent in the

stranger chamber, suggesting that if the mouse had a positive R score, it spent a larger percent of time with the stranger. For each experimental mouse, social cognitive preference comparisons were made for how much time the mouse spends in either the chamber with the littermate versus the novel mouse, and additionally if there are any differences in the time the mouse spent engaged in direct versus peripheral interaction within its preferred chamber. These data were collected at PND 30 and PND 60 for the same mice in both treatment conditions and compared for differences between groups, but also for the same animal at two separate time points.

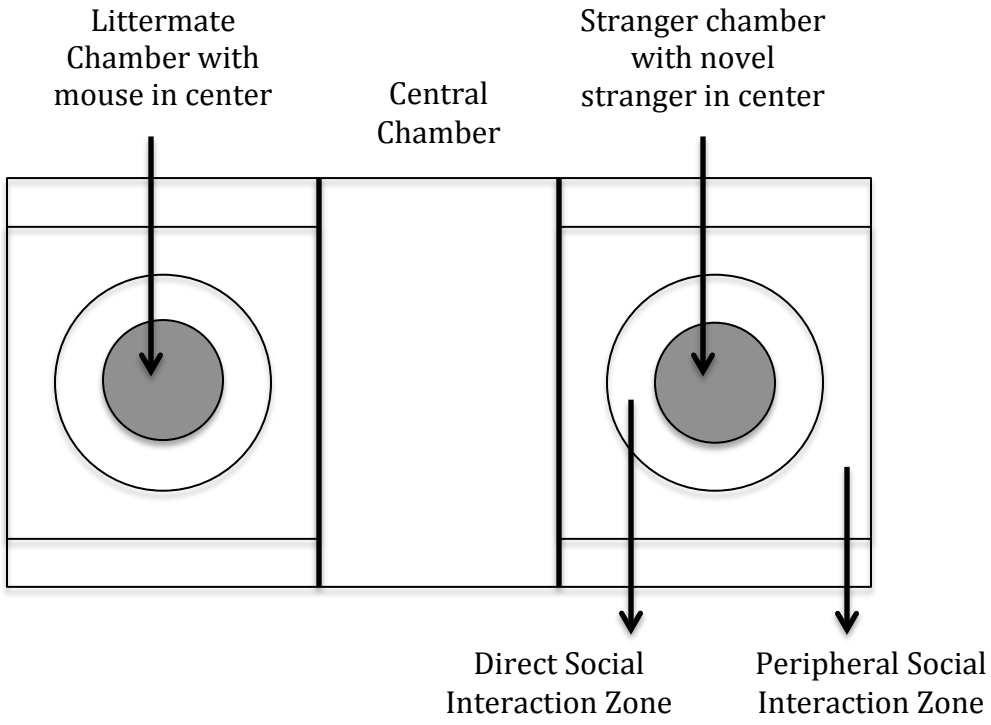

Figure 2- Arena Tracking settings for Behavioral Analysis using EthoVision
Division of behavioral arena into chambers, subdivided into interaction zones that provide further qualitative information about the nature of the social interaction the animal engages in. More time spent in direct interaction suggests that the animal is more likely to have typical social recognition and memory versus an animal that spends more time engaging in peripheral interaction.

Statistical Analysis

For behavioral analyses, social recognition data were analyzed using SPSS 21. To determine the baseline behavior in the altered social recognition paradigm using control C57 mice, a paired samples t-test was used to determine whether the same mouse spent different amounts of time in the direct stranger and direct littermate zones, versus peripheral stranger and peripheral littermate zones. These data were also analyzed for any potential interactions.

To study the effects of maternal immune activation on social recognition behavior, statistical analyses were conducted separately for the three cohorts. For the first cohort, a 2 (Poly I: C, Saline) x 2 (Littermate chamber, Novel mouse chamber) x 2 (Direct Social Interaction, Peripheral Social Interaction) mixed model ANOVA was used to compare time spent in either chamber, and additionally time spent in the peripheral versus direct interaction zones in relation to treatment. The chamber preference was considered a within- subject variable that was then compared between treatment conditions. The R (recognition) scores, average amount of distance travelled and mean velocity were analyzed individually (each considered a within subjects variable) using an independent samples t- test between treatment groups.

To study interaction zone choice at PND30 in the second cohort, a 2 (Poly I: C, Saline) x 4 (Direct Stranger, Peripheral Stranger, Direct Littermate, Peripheral Littermate) Repeated Measures ANOVA was used, where interaction zone choice was considered the within subjects variable and analyzed for

differences between treatment conditions. Similarly, a 2 (Poly I: C, Saline) x 2 (Stranger, Littermate) Repeated Measures ANOVA was used to analyze treatment-based differences in chamber preference (considered the within-subjects variable). The R Score, mean distance and mean velocity (considered within-subjects variables) were analyzed using an independent samples t-test. At PND60, a 2 (Poly I: C, Saline) x 4 (Direct Stranger, Peripheral Stranger, Direct Littermate, Peripheral Littermate) Repeated Measures ANOVA was used to determine interaction zone preference. Again, a 2 (Poly I: C, Saline) x 2 (Stranger, Littermate) Repeated Measures ANOVA was used to determine how treatment affected chamber preference.

These data were then combined to determine how maternal immune activation impacted social recognition and memory between when animals were juveniles and adults. A 2 (Poly I: C, Saline) x 2 (PND30, PND60) x 2 (Stranger, Littermate) mixed model ANOVA was used to determine differences in chamber preference (considered a within-subjects variable) between treatment groups. Similarly, a 2 (Poly I: C, Saline) x 2 (PND30, PND60) x 4 (Direct Stranger, Peripheral Stranger, Direct Littermate, Peripheral Littermate) Repeated Measures was also conducted to determine interaction zone preference. For both the above analyses, the treatment condition was considered a between subjects variable, while the chamber preference, interaction zone choice and age were considered within-subjects variable. Mean distance and velocity were analyzed using a 2 (Poly I: C, Saline) x 2 (PND30 distance, PND 60 distance) or 2 (Poly I: C, Saline)

x (PND30 velocity, PND60 velocity) Repeated Measures ANOVA respectively. Similarly, R Scores were analyzed using a 2 (Poly I: C, Saline) x 2 (PND30 R Score, PND60 R Score) Repeated Measures ANOVA. In these analyses, mean distance travelled; mean velocity and R scores were considered within- subjects variables that were analyzed between treatment conditions.

With the third cohort, analyses of covariance were conducted to further understand how social memory might be developing between when the animals were juvenile to when they were adults. Animals were also compared between treatment conditions to determine their ability to retain a social memory long term by testing with same littermate pairings as PND30 at PND60. Additionally, social recognition based on the ability to develop social memory in a short, 10-minute exposure to a novel animal was determined when animals showed social preference between an acquainted stranger versus a novel stranger mouse. Long-term memory retention was determined based on the amount of time spent by the experimental animal in each chamber. It was hypothesized that typically developing animals with intact social recognition and memory would spend equal time with both the stranger and littermate mice at PND60. Alternatively, typically developing animals would spend more time in the chamber with the novel stranger versus the acquainted stranger. Analyses for long-term versus short-term memory and recognition were conducted using a 2 (Poly I: C, Saline) x 2 (Stranger, Littermate) ANCOVA with chamber preference at PND30 between treatment conditions as a co-variate. Similarly, a 2 (Poly I: C) x 4 (Direct Novel

Stranger, Peripheral Novel Stranger, Direct Acquainted Stranger, Peripheral Acquainted Stranger) ANCOVA with zone preference at PND30 between treatment conditions as a covariate was also conducted to determine whether novelty preference changed between ages.

Additionally, to determine any differences in mean distance travelled or average velocity, a 2 (Poly I: C, Saline) x 2 (PND30 mean distance, PND60 mean distance) and a (Poly I: C, Saline) x 2 (PND30 mean velocity, PND60 mean velocity) Repeated Measures ANOVA was used respectively. Additionally, R scores were also analyzed using a 2 (Poly I: C, Saline) x 2 (PND30 R Score, PND60 R Score) Repeated Measures ANOVA.

All analyses were two- tailed with $p < 0.05$ considered as statistical significance.

RESULTS

The following study was conducted to initially determine baseline social interaction preference in an altered social recognition paradigm in C57 mice based on what zones (i.e. direct or peripheral) these mice spent the most time in as a measure of engagement with the novel or familiar social stimuli. Next, this paradigm was tested with separate treatment groups to ascertain differences in social recognition behavior that might be mediated by maternal immune activation, implying deficits in social recognition and memory. Comparisons for interaction zone choice, chamber preference, recognition scores (R Scores), distance and velocity were compared across treatment groups at PND30 and PND60, and then combined to establish changes in social recognition in the same experimental animal over time. Additionally interaction zone preference was also analyzed at PND60 for novelty preference as a measure of social recognition and memory. The treatment conditions (Poly I: C versus saline) and the age (namely PND30 and PND60) were considered independent variables, while all other measured variables were dependent variables.

Altered Social Recognition Paradigm

A paired samples t- test was used to determine whether adult C57 mice had any interaction zone (direct littermate, peripheral littermate, direct stranger and peripheral stranger) preference specific to either the stranger or littermate chamber. A significant main effect was detected for direct interaction with the

novel stranger mouse, t (16)= 1.68, p= 0.038, indicating that these mice spent more time in direct engagement with the novel mouse, as expected in species typical behavior (Fig 3).

Effect of MIA at PND60

The following analyses were conducted in adult C57 mice to determine if MIA mediated any behavioral differences in the altered social recognition paradigm. Analysis of interaction zone preference revealed a zone choice by treatment interaction that trended to significance, F (1,3) =1.965, p = 0.134, *partial eta squared* = 0.123. However, there was no main effect for treatment condition, F (1,3) = 0.623, p=0.443, *partial eta squared* =0.043. This shows that C57 mice spent most time in direct interaction with the stranger, and least time in peripheral interaction with the littermate across treatment groups. Post hoc analyses using independent samples t-tests between treatment conditions for each zone showed that saline mice spent more time with in direct interaction with the novel stranger than the familiar littermate mouse, t (8.104) = 2.032, p = 0.076, and this difference trended to significance (*Fig 4a*). These findings in conjunction suggest that MIA is mediating a social recognition deficit in adult C57 mice, causing Poly I: C offspring to spend significantly less time in direct interaction with the novel stranger mouse.

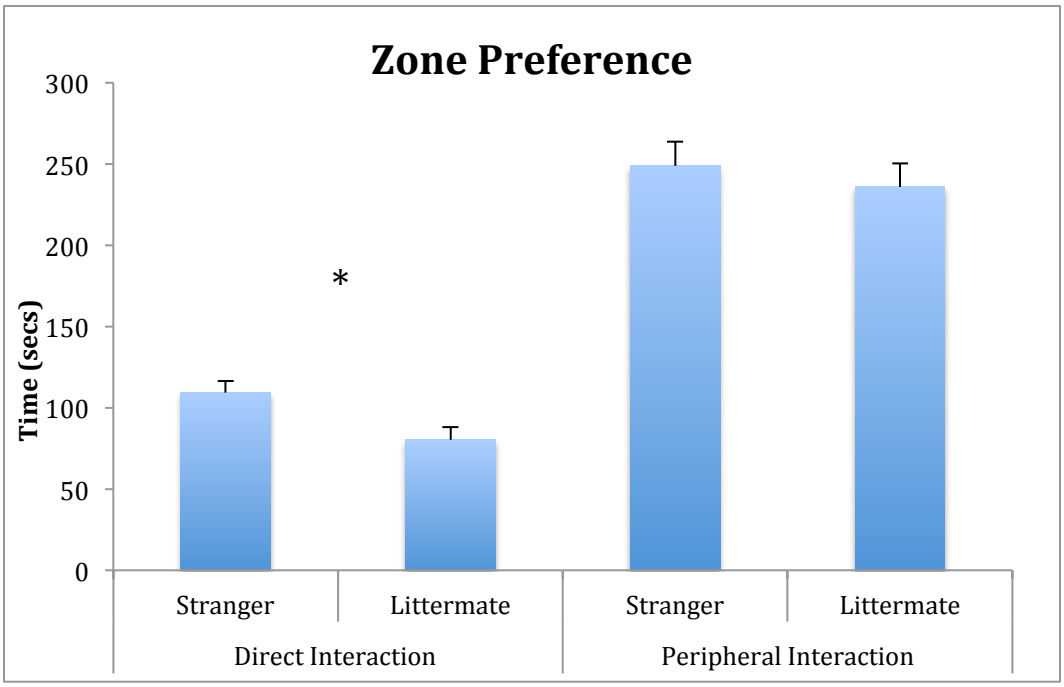

Figure 3- Zone Preference using Altered Social Recognition Paradigm. This analysis was conducted using adult untreated C57 mice to validate this paradigm. C57 mice were found to be spending more time in direct interaction, and spent significantly more time directly interacting with the stranger mouse versus the littermate mouse, $p = 0.038$.

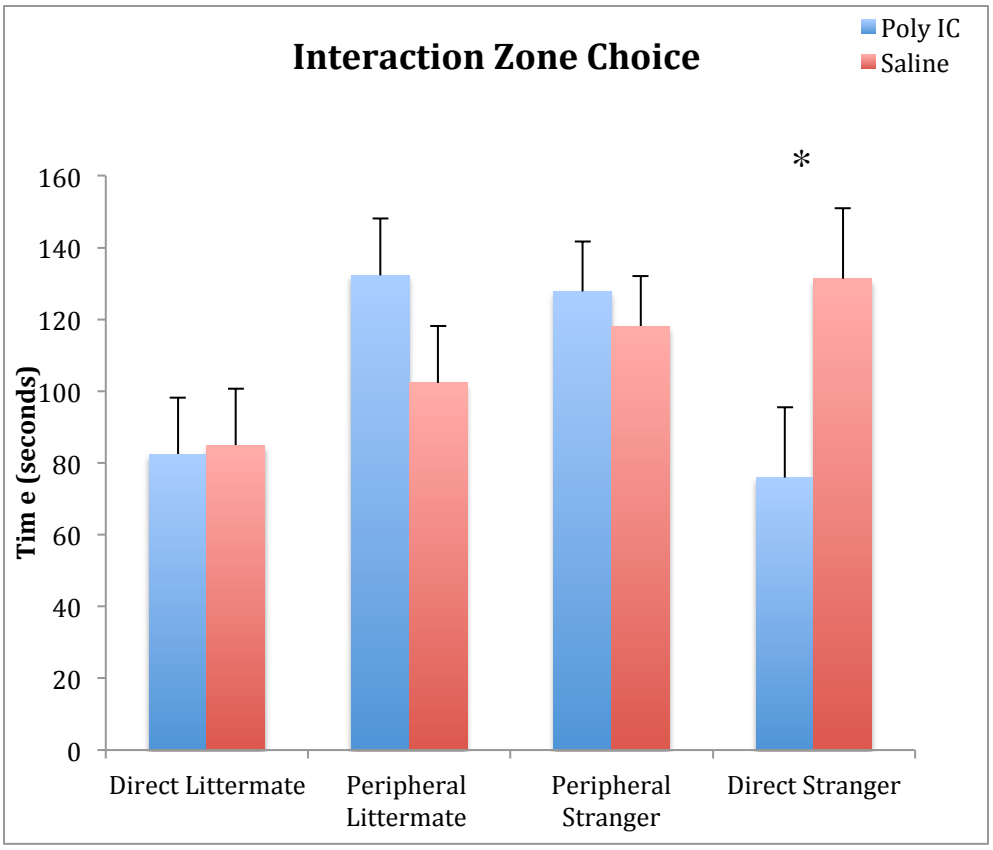

Figure 4a- Interaction Zone Preference between Treatment Groups at PND60

At PND60, saline offspring spent significantly more time in direct interaction with the novel stranger mouse than Poly I: C offspring, p= 0.076. There were no differences between treatment groups for the other interaction zones.

Further analyses using whole chamber preference data i.e. time the animal spent in the chamber, collapsing across interaction zones revealed that mice across treatment groups were spending more time in the chamber with the novel stranger mouse, F (1,1) = 2.259, p= 0.154, *partial eta squared*= 0.131 (Fig 4b), though this difference was only a trend to significance. There were also no chamber preference by treatment interaction effects, F (1,1) = 1.634, p=0.221, *partial eta squared* = 0.098 or main effects for treatment observed, F (1,1)= 0.236, p= 0.634, *partial eta squared*= 0.015. These findings suggest that though both Poly I: C and saline mice were both spending more time in novel stranger chamber overall, the Poly I: C mice were not spending time directly engaging with the stranger mouse. The displayed chamber preference along with the interaction zone choice suggest that MIA might be impacting the ability of C57 mice to engage in direct social interactions as adults, implying deficits to social recognition (Fig 4a, 4b).

Furthermore, an independent samples t- test was used to determine differences between treatment groups for R scores (or the difference in percent of time the experimental mouse spent in the stranger versus littermate chamber, where a positive score indicates more time spent in the novel stranger chamber). There were no significant differences in the percent of time spent in either chamber across treatment groups, t (15) =1.298, p=0.221.

This suggests that though both groups spent equal percent of their time exploring both chambers, Poly I: C offspring lacked the direct social interaction ability to engage in social recognition as adults.

Additionally, independent samples t- tests were used to ensure that there were no main effects for treatment conditions with regard to mean distance travelled and mean velocity during the time of the test session. Differences in these variables can offer different potential explanations to the observed differences in social recognition, like treatment-based differences in motor performance. No main effects for neither distance, t (7.142) = 1.036, p = 0.334 (Fig 5a), nor velocity, t (7.153) = 1.027, p=0.338 (Fig 5b) between treatment groups were found. This further confirms that the MIA mediated differences in social recognition are not confounded by differences in locomotor performance.

This first set of findings establish that MIA mediates social recognition deficits in Poly I: C offspring once they adults, or at PND60. A new cohort was used to determine whether these treatment-based differences in social recognition exist when animals are juveniles i.e. at PND30, and whether these differences sustain into adulthood.

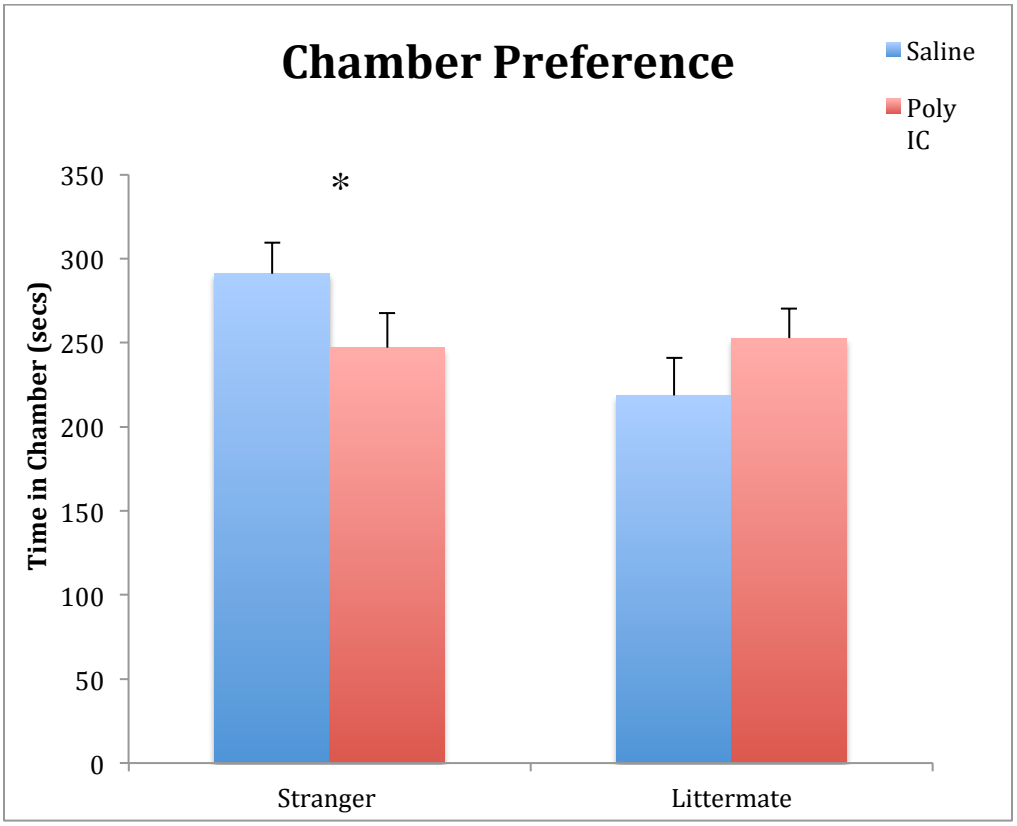

Figure 4b- Chamber Preference between Treatment Groups at PND60
Both treatment groups spent more time in the stranger chamber than littermate chamber, though this difference trended to significance, p=0.15. Findings suggest there might be specificity to a particular interaction zone with the novel stranger chamber that reflects the differences in performance in social recognition ability (shown as the chamber by treatment interaction in Fig 4a).

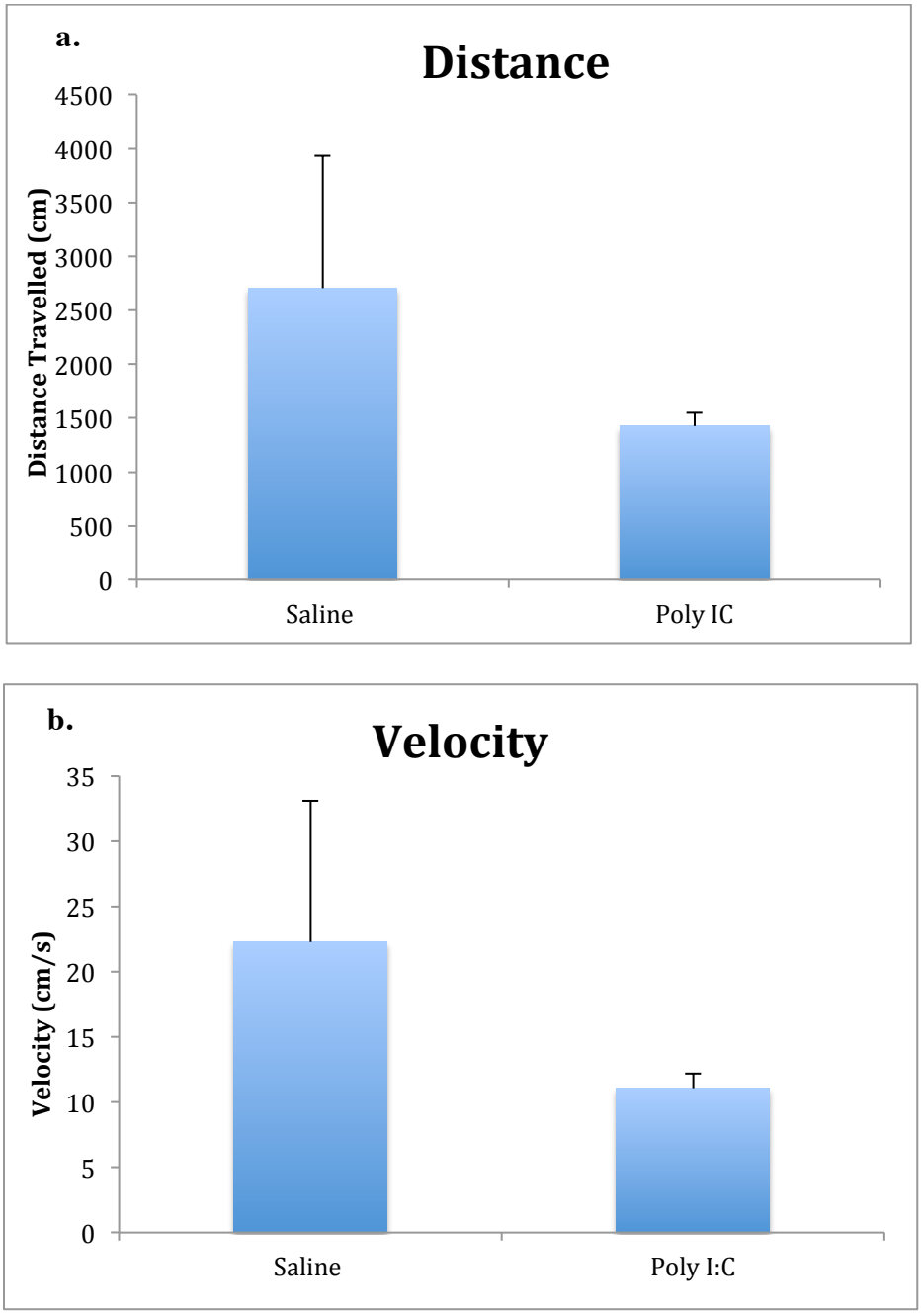

Figure 5- Measures of Locomotor Performance between Treatment Groups. **a.** Graph showing average distance travelled during the test period; there was no significant difference between treatment conditions, p= 0.334. **b.** Graph showing mean velocity during the test period; similarly, there was no significant difference between treatment conditions, p= 0.338. However, both variables show a high amount of variability in both in distance and velocity in saline offspring.

Effects of MIA on Social Recognition at PND30

Analyses were conducted to determine whether interaction zone preference (namely direct stranger, peripheral stranger, direct littermate, peripheral littermate; *see Figure 1 for details*) between treatment conditions followed the same trend in juveniles as was established in adults. There was a significant main effect for zone preference, F (1,3) = 6.482, p= 0.001, *partial eta squared*= 0.206. Mice across treatment groups spent the most time in direct interaction with the novel stranger mouse (*Mean* = 145.700, *MSE*= 10.906), equal time interacting peripherally with the stranger mouse (*Mean*=106.392, *MSE*=8.615) and in direct interaction with the littermate mouse (*Mean*=109.411, *MSE*=9.565), and the least time interacting peripherally with the littermate (*Mean*=85.917, *MSE*=5.170) (Figure 6a). However, a significant zone preference by treatment interaction was not revealed at PND 30, F (1,3) = 1.006, p= 0.395, *partial eta squared*= 0.039 and there was also no main effect between treatment groups, F (1,3)= 0.147, p= 0.705, *partial eta squared*= 0.006. Post hoc analyses using independent samples t-tests for each interaction zone revealed no significant differences between treatment groups (Direct Stranger, t (19) =0.667, p =0.513; Peripheral Stranger, t (19) = 0.915, p= 0.372; Direct Littermate, t (19) = 0.479, p=0.638; Peripheral Littermate, t (19) =0.515, p=0.613). These data only partially agree with previous findings from adult mice, suggesting that C57 mice at PND30 across treatments do not spend the most time in direct interaction with the novel stranger mouse.

However, there was a significant main effect for chamber preference at PND30, F (1,2)=74.841, p=0.000, *partial eta squared*= 0.75, where again mice across treatment groups showed a strong social preference for the chamber with the novel stranger mouse (*Mean*= 281.753, *MSE*=10.761). A chamber preference by treatment interaction that trended to significance was also revealed, F (1,2) = 2.972, p=0.06, *partial eta squared* = 0.106 (Fig 6b), where saline offspring spent significantly more time interacting with the novel stranger mouse than the littermate mouse, while Poly I: C offspring spent equal amount of time interacting with both the stranger and littermate mouse. However, there was no significant main effect for treatment, F (1,2) =0.825, p=0.373, *partial eta squared* = 0.032. These data suggest that MIA is mediating differences in social recognition in Poly I: C offspring as early as PND30, resulting in these offspring not displaying a chamber preference.

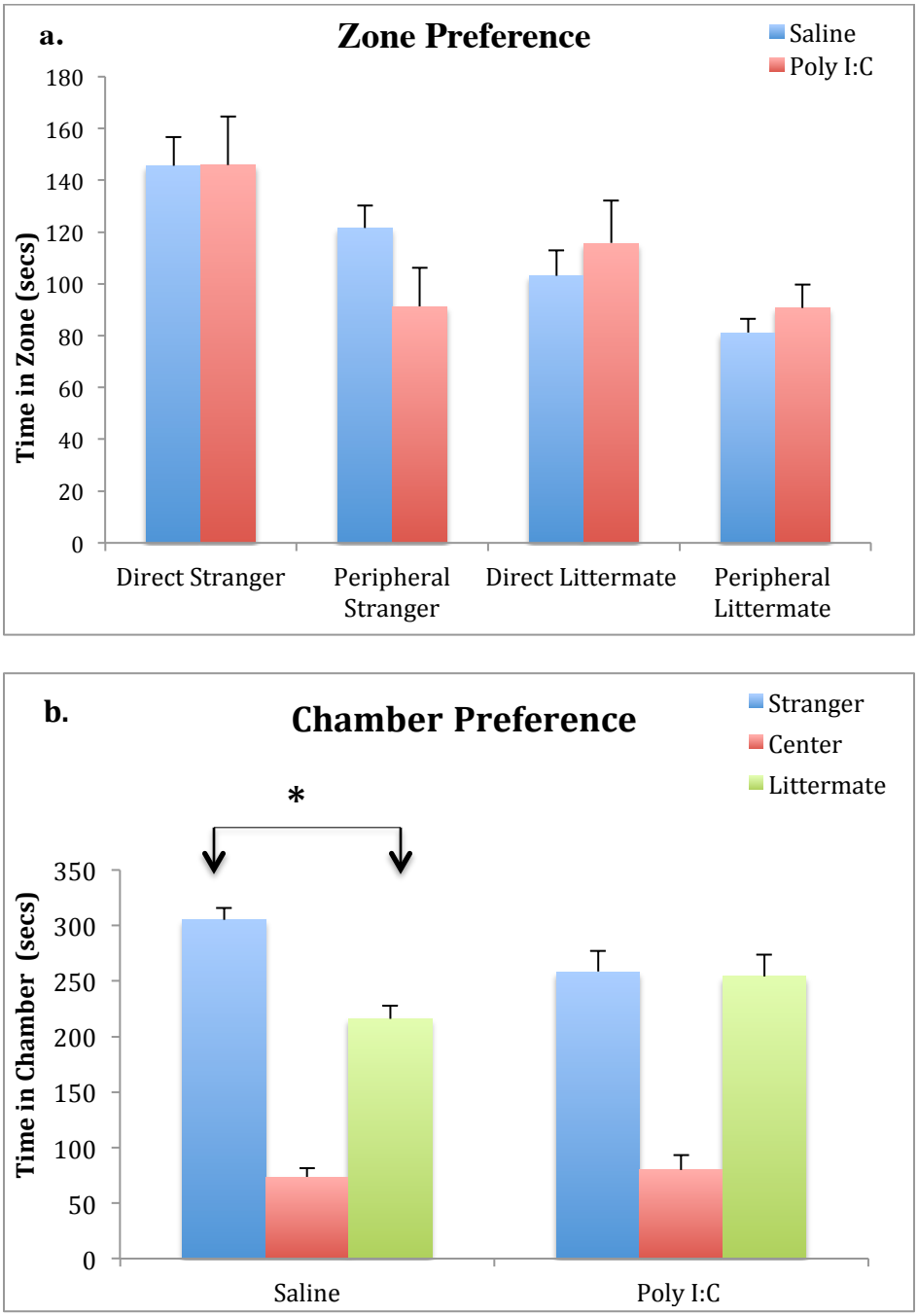

Figure 6- Zone Preference and Chamber Preference between Treatment Groups at PND 30.
a. Data showing that neither Poly I: C nor saline offspring show an interaction zone preference at PND30. **b.** Data showing a significant chamber preference by treatment interaction (p= 0.06), where saline offspring spend more time interacting with the stranger mouse than littermate mouse, while Poly I: C offspring spent equal time interacting with both the stranger and littermate mouse.

R scores were analyzed using an independent samples t- test, and there were no significant differences between treatment groups, t (19)= 0.624, p=0.54. This further indicates that similar to social recognition differences between treatments at PND60, neither Poly I: C nor saline offspring spent significantly different percentages of the total test time exploring either chamber. This further indicates that the inability of Poly I: C to show a chamber preference during the total test time is not attributed to them spending a different amount of time in each chamber. Assessments of locomotor activity were also analyzed using independent samples t- tests; there were no significant differences between treatment conditions for neither mean distance travelled, t (19) = 1.112, p=0.221 nor velocity, t (19) = 0.671, p= 0.510. This indicates that differences in motor activity do not provide an alternate explanation for the deficits in social recognition seen in Poly I: C offspring compared to saline offspring at PND30.

Effects of MIA on Social Recognition at PND60

When data from the same animals were analyzed at PND60, there was no significant main effect for interaction zone choice, F (1, 3)=0.264, p =0.851, *partial eta squared*= 0.014, or interaction zone by treatment interactions, F (3)=0.422, p=0.738, *partial eta squared*= 0.022. There was also no main effect for treatment, F (1)=0.460, p=0.506, *partial eta squared*=0.024. Marginal means suggest that at PND60, Poly I: C offspring spent more time in direct interaction with the novel stranger mouse (*Mean*= 129.788 seconds, *MSE*= 20.085) than in

direct interaction with the littermate mouse (*Mean*=102.955 seconds, *MSE*=36.324). Alternatively, saline offspring spent almost equal amount of time in direct interaction with both the novel stranger (*Mean*= 101.668 seconds, *MSE*=12.703) and the littermate mouse (*Mean*=103.530, *MSE*=22.973), though these differences were not significant. These data suggest a causative relationship between Poly I: C mediated MIA and behavioral deficits in social recognition in mice. Similarly, there were no significant main effects for chamber preference, F (1)=0.389, p=0.56, *partial eta squared*=0.02, or chamber preference by treatment interactions, F (1)=0.553, p=0.466, *partial eta squared*=0.023. Between subjects analysis revealed a main effects for treatment conditions that trended toward significance, F (1)=2.780, p=0.112, *partial eta squared*=0.128, where Poly I: C mice were found to spend more time engaging with the stranger mouse, implicating that MIA mediated social recognition deficits do change slightly between PND30 and PND60, yet Poly I: C offspring fail the social recognition task at both ages suggesting a behavioral deficit.

Again, differences in R Scores between treatment groups were analyzed using an independent samples t- test, and there were no significant differences in the percentage of time either treatment condition spent exploring the novel stranger versus littermate chamber, t (19)= -0.677, p=0.506. However, Poly I: C offspring had more positive scores (*Mean*= 0.1581, *MSE*=0.0645) than saline offspring (*Mean*= -0.0028, *MSE*= 0.0780) on average, indicating that they spent a larger percent of time interacting with the novel stranger mouse at PND60. There

were no differences in motor abilities between treatment groups, namely distance, F (1)=0.651, p=0.430, *partial eta squared*=0.033, and velocity, F (1)=0.547, p=0.469, *partial eta squared*=0.028, ruling out differences in locomotor ability as a possible explanation for these results.

Effects of MIA on Social Recognition between Age and Treatment Groups

Data from the same experimental pairings were combined and longitudinally analyzed as an additional within-subjects repeated measures variable. This analysis was conducted to determine whether social recognition ability changed significantly between when the same animal was a juvenile versus adult, and further analyzed for differences between treatment groups.

There were no significant within-subject interactions for neither interaction zone preference by age by treatment, F (1,3) = 0.911, p=0.442, *partial eta squared*=0.046 or zone preference by age, F (1,3) =1.131, p= 0.344, partial eta squared = 0.056. There was also no main effect for treatment group at either age, F (1,3) =0.388, p=0.541, *partial eta squared*= 0.020. This indicates that the hypothesized zone preference by treatment interaction was not significant enough at any one given developmental age, such that it would be significant in a combined analysis.

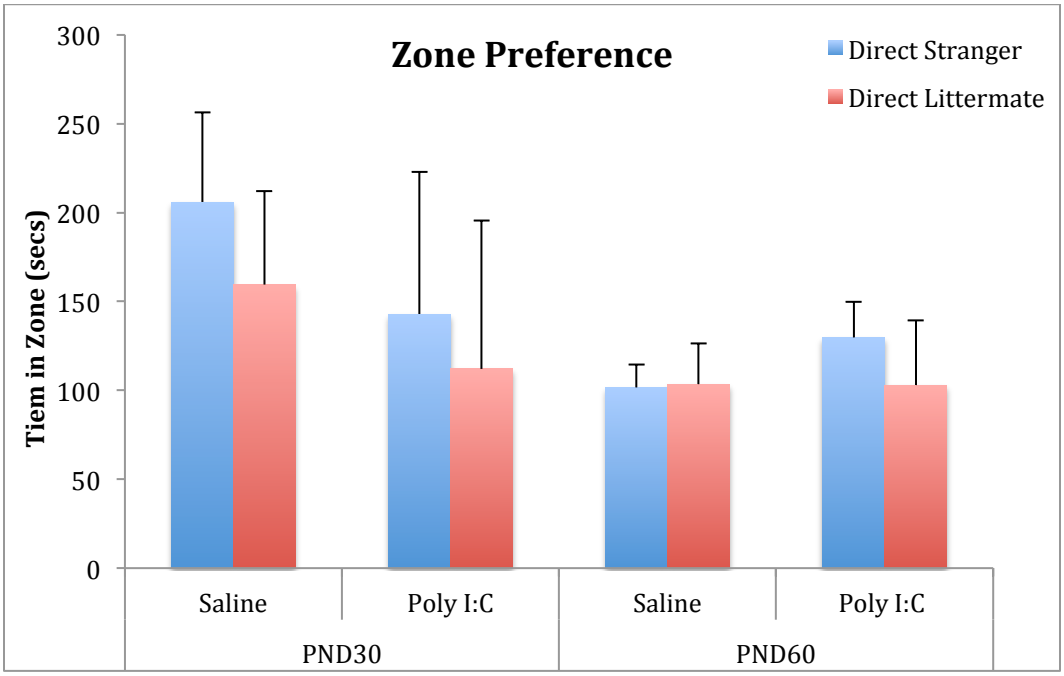

Figure 7- Interaction Zone Preference by Treatment by Age Interaction. Graph showing no differences in interaction zone preference between Poly I:C and saline offspring, at both PND30 and PND60.

However, an analyses of chamber preference between treatment groups, while also between PND30 and PND60 in each individual animal revealed a significant chamber preference by treatment by age interaction, F (1,3)= 3.205, p=0.089, *partial eta squared*=0.144. These data indicate that Poly I: C mice at PND 30 showed no chamber preference between stranger and littermate, but at PND 60, spent significantly more time in the stranger chamber. Conversely, saline mice spent significantly more time in the stranger chamber at PND 30, but showed no difference between times spent in either chamber at PND 60. This indicates that not only does MIA mediate social recognition deficits in juvenile Poly I: C mice, but also these deficits persist into adulthood (Fig 8).

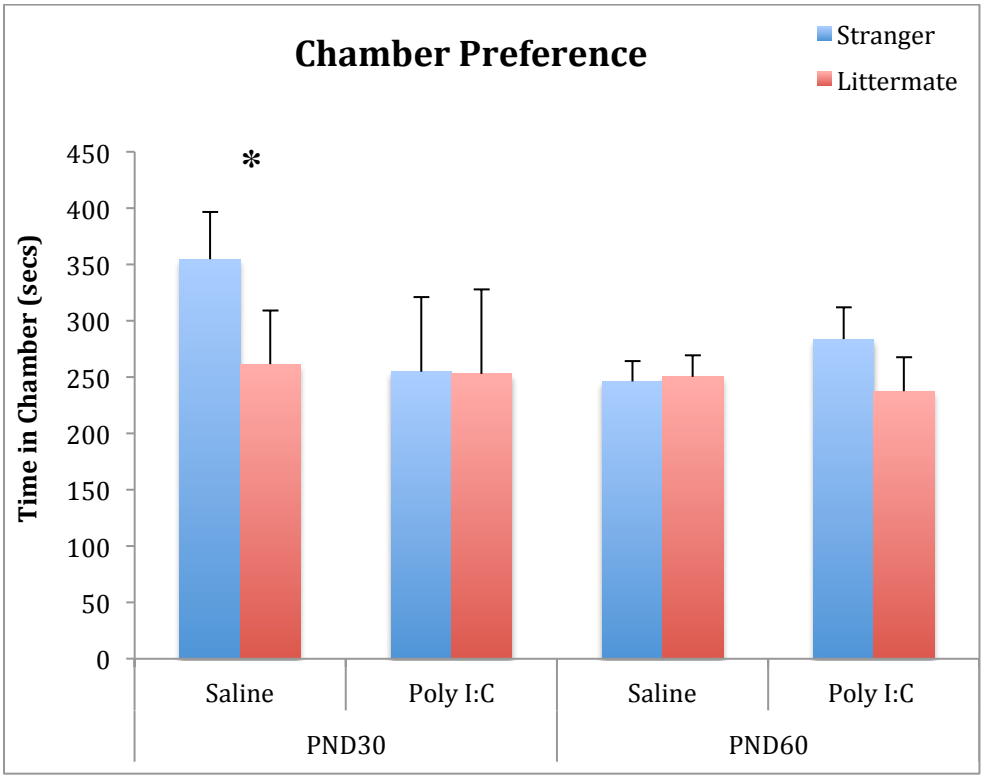

Figure 8- Age by Treatment by Chamber Preference Interaction
Graph showing how MIA mediates social recognition deficits between ages. At PND30, saline mice spent significantly more time in the stranger chamber versus the littermate chamber, but at PND60, they did not show any such preference. Poly I: C offspring spent equal amount of time in both the stranger and littermate chamber at PND30, while at PND60, they spent more time in the stranger chamber. However, this difference at PND60 was not significant. This indicates how MIA causes social recognition deficits in C57 mice that start when they are juveniles and persists into adulthood.

Further analyses conducted with R score data showed no significant interaction between R Scores, treatment and age, F (1,1)=0.391, p=0.539, *partial eta squared*=0.020 (Fig 9). Furthermore, there were also no significant treatment effects, F *(1,1)*=0.389, p=0.540, *partial eta squared*= 0.020, indicating that there were no differences in the percent of time both Poly I: C and saline mice spent preferentially in the novel stranger chamber when compared across age. Similarly, combined analyses ruled out differences in motor performance as a potential confound. There were no significant main effects across age for neither distance, F (1)=0.227, p=0.639, *partial eta squared*=0.012 (Fig 10a), nor velocity, F (1)=0.363, p=0.554, *partial eta squared*=0.019 (Fig 10b). There were also no main effects for treatment reflected in measures of distance, F (1)=2.110, p=0.163, *partial eta squared*= 0.100, or velocity, F (1)=0.542, p=0.471, *partial eta squared*=0.028.

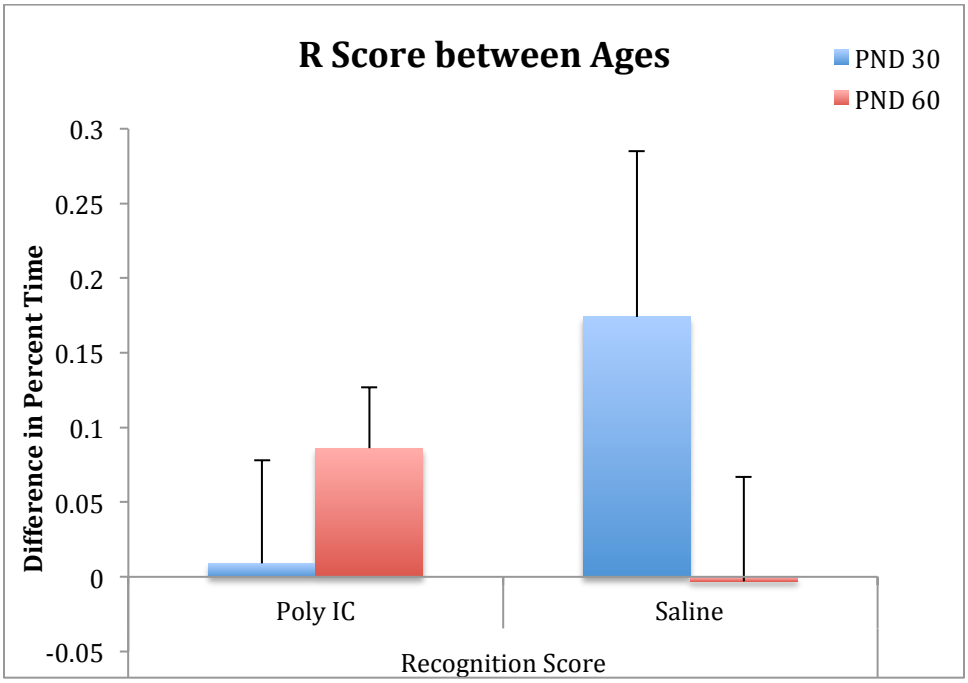

Figure 9- R Score by Treatment by Age Interaction
Graph showing how the effects of MIA on R scores between ages. Neither saline nor Poly I: C offspring spent a significantly higher percent of the total test time in the stranger chamber at both PND30 and PND60.

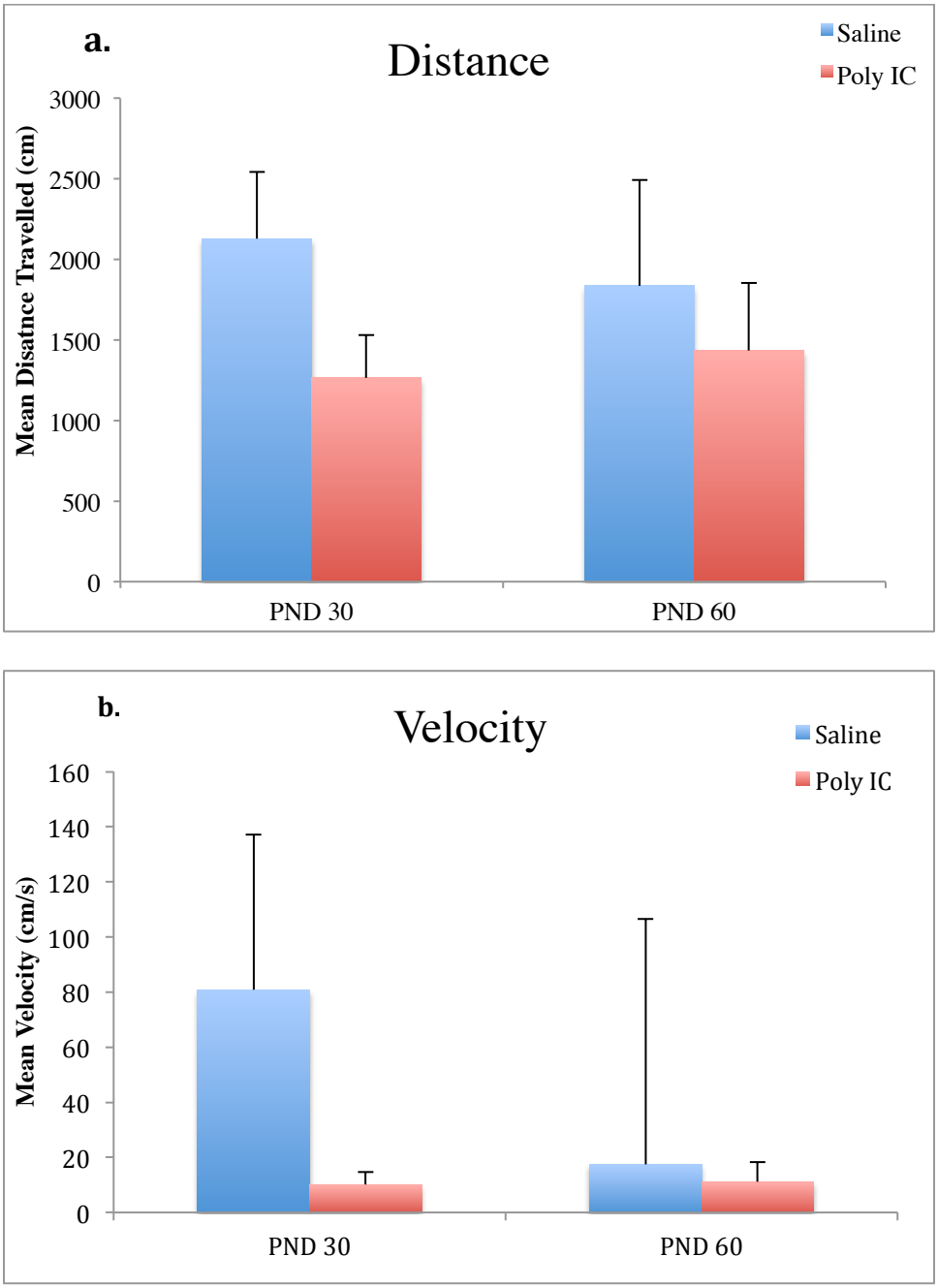

Figure 10- Analyses of Locomotor Performance between Ages.
a. Graph showing no differences in mean distance travelled between Poly I: C and saline offspring between ages. **b.** Graph showing no differences in mean velocity between treatment groups and both at PND30 and PND60.

Another cohort of animals was used to replicate the social recognition deficits found in Poly I: C mice between ages in the previous cohort, and to determine the potential role of social memory in social recognition deficits. An analysis of zone preference by treatment by age revealed no significant interaction effects, F (1,3)=0.424, p=0.736, *partial eta squared*= 0.018. These results are consistent with the findings from the previous cohort, where the zone preference between Poly I: C and saline mice was not found to be significantly different between PND30 and PND60.

Conversely, repeated measures analyses were used to replicate the findings of the previous chamber preference by treatment by age interaction, which showed that Poly I: C offspring did not spend significantly different amounts of time in both the stranger and littermate chambers at both PND30 and PND60, indicating social recognition deficits. However, a replication study revealed no significant interaction, F (1,2)=0.018, p=0.982, *partial eta squared* = 0.001 (Fig 11). Data from this cohort suggest that MIA does not mediate social recognition deficits in Poly I: C offspring between ages; alternatively, there seems to be no effect for the treatment suggesting that the effects obtained in the previous cohort could be by random chance. An ANCOVA was conducted to determine how the chamber preference changed between treatment groups relative to the individual animal's chamber preference at PND30. A main effect for treatment trended to significance, F (1,2) =2.160, p=0.157, *partial eta squared* = 0.097. This suggests that MIA changes chamber preference between PND30

and PND60; however, these results should be interpreted with caution as no significant differences were detected relative to the set alpha value.

Also consistent with data from the previous cohort, there were no differences in R Scores between treatment groups at PND30 and PND60, F (1,1)=0.00, p=0.991, *partial eta squared*=0.000. This indicates that both Poly I: C and saline offspring spent equal percentage of the total test time exploring both the stranger and littermate chamber. Similarly, there were no treatment-based differences in mean distance travelled, F (1,1)=0.447, p=0.510, *partial eta squared*=0.010 or mean velocity, F (1,1)= 1.303, p=0.265, *partial eta squared*= 0.054. These data further ascertain that both Poly I: C and saline offspring in this cohort were matched in locomotor performance

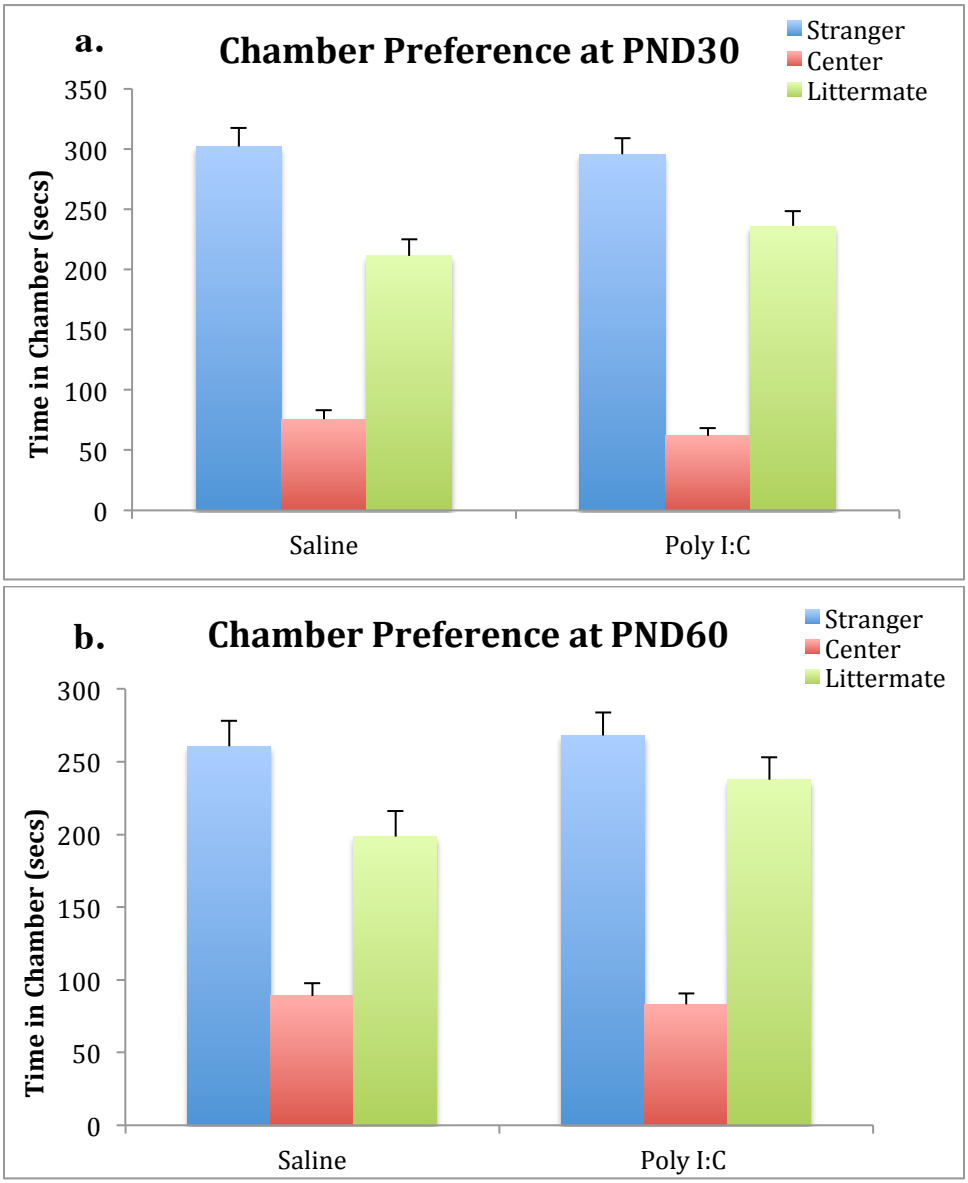

Figure 11- Replication Study for Chamber Preference by Treatment by Age Interaction.

a, b. Graphs showing data for chamber preference by treatment interaction at PND30 and PND60 respectively. There were no significant differences between treatment conditions.

At PND60, the same animals were used to also determine chamber and interaction zone preference between an acquainted stranger mouse and a novel stranger mouse. It was hypothesized that typically developed (i.e. saline) offspring would associate the acquainted stranger mouse with the littermate mouse as they have met the same animal greater than one time, and show a preference for the novel stranger mouse. Analyses of zone preference for either the novel stranger versus acquainted stranger chamber between treatment groups at both PND30 and PND60 showed no significant interaction effects, F (1,3) = 1.408, p=0.248, *partial eta squared*=0.058. An analysis of covariance for zone preference within the acquainted versus novel stranger chambers using chamber preference at PND 30 as a covariate revealed a treatment by zone preference interaction that trended to significance, F (1, 3) = 2.359, p=0.141, *partial eta squared*=0.110 (Fig 12). These data indicate that at PND60, Poly I: C offspring spent significantly more time in direct interaction with the novel stranger versus the acquainted stranger than saline offspring. This further suggests that MIA did not cause social recognition deficits in Poly I: C offspring, as they display a preference for novel social interaction.

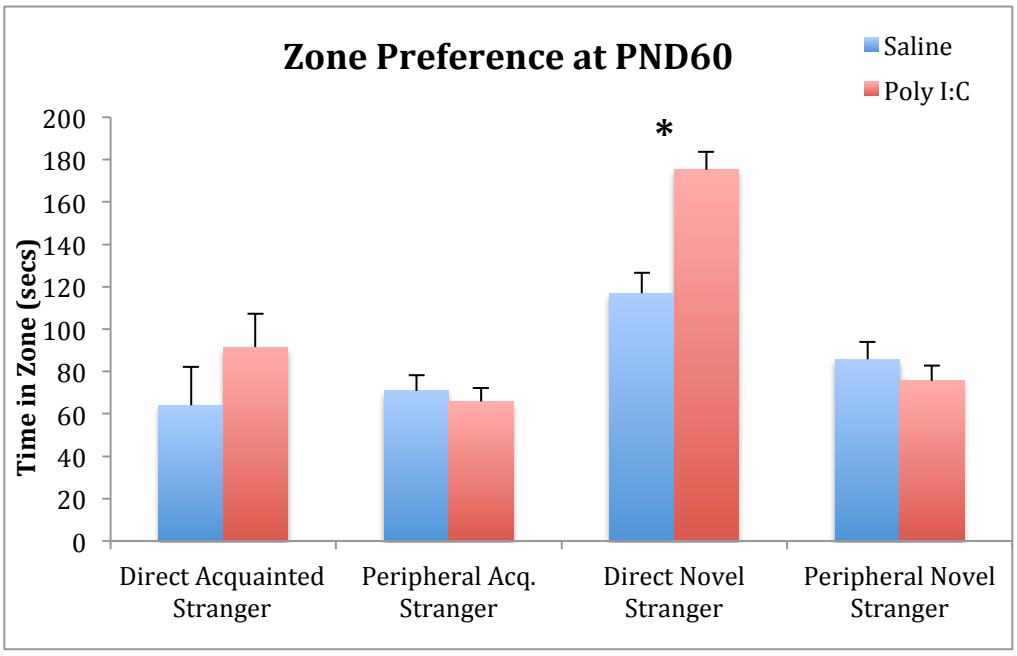

Figure 12- Analysis of Covariance at PND60 in Zone Preference between Treatment Groups.
Graph showing that in comparison to mean zone preference for each interaction zone between treatments at PND30, Poly I: C offspring spent significantly more time than saline offspring in direct interaction with the novel stranger versus acquainted stranger mouse at PND60.

DISCUSSION

Interpretation of Results

This study examined the effects of maternal immune activation on the development of social recognition in mice, and found that prenatal exposure to an elevated immune response in the mother mediates neurodevelopmental deficits in social cognitive processing in the offspring. C57 mice used to establish a species typical social recognition behavioral baseline were found to be more likely to be social by spending significantly longer time directly engaged in direct interaction with the novel mouse. Furthermore, a similar trend was observed in saline mice at PND30 and PND60; these mice spent most time in direct interaction and showed a strong chamber preference at PND30 for the novel stranger mouse over the littermate mouse. At PND60, saline mice showed neither interaction zone preference nor chamber preference, suggesting that they did not inherently consider either of these social stimuli to be different. This further illustrates that a short one time exposure to the stranger mouse at PND30 was enough for social memory formation in saline offspring evidenced in their lack of chamber preference at PND60. This is in sharp contrast to Poly I: C offspring who do not show social recognition via having a chamber preference at PND30, and consequently do not show a chamber preference at PND60 either. This points towards the importance of the symbiotic relationship between social recognition and memory in mediating the development of appropriate social interaction through age.

The trends of sociability for Poly I: C offspring differed in interesting ways from saline mice. Poly I: C pups at PND30 spent equal time in direct interaction with the novel stranger and familiar littermate. This points toward an inherent recognition deficit where these mice are unable to tell the difference between the novel and familiar mouse. At PND60, Poly I: C mice do spend more time in the direct interaction zone with the stranger mouse (this difference was not significant), followed by the peripheral interaction zone in the stranger mouse chamber. This shows that though Poly I: C offspring seem to be developing social recognition at PND60 and try to initiate social interaction with the novel mouse, they still preferentially engage with their littermate. Furthermore, they are still not able to succeed in the task implying that MIA causes social recognition deficits in C57 mice that are prevalent from juveniles and sustained into adulthood. This trend in social behavior is reminiscent of cognitive deficits in comparison to typically developing saline mice.

This similar trend is displayed in chamber preference for Poly I: C offspring, where at PND30 they show no chamber preference, while at PND60, they spent significantly more time interacting with the stranger mouse. These effects suggests that these mice did not have developed social recognition and memory skills at PND30, and hence show neither an interaction zone nor a chamber preference. This further indicates a causative relationship between maternal immune activation via viral pathogens and developmental delays in cognition and memory linked to social behavior. When these findings are

considered with respect to clinical symptoms observed in patients with autism, impairments in eye gaze and inability to comprehend mental states and intentions are often considered key developmental differences in these patients in comparison with typically developing age matched individuals. This process of social cognitive development dependent on input from a primary sensory modality has to be adapted to mice in order to understand how behavioral tasks like social recognition effectively model MIA- mediated socio- cognitive deficits. Human beings perceive sensory stimuli primarily via their eyes; furthermore, Pelphrey and colleagues (2005) conducted an event- related fMRI study with autistic and typically developing individuals and found that autistic individuals could not appropriately differentiate between congruent and incongruent eye gaze shifts linked to social stimuli. This further supports the argument that social behavior deficits central to autism and other neurodevelopmental conditions are strongly linked to recognition ability. For C57 mice, since these animals are not visually dominant and encode their social stimuli primarily via smell, their sociability can be measured by their preference for novel social scents, such as those of a stranger mouse they have never interacted with before.

Limitations

Additionally, studies involving clinical memory tests administered to individuals with high functioning autism in comparison with typically developing age matched controls found differences reflected as poor memory for complex

visual and verbal information, with the most significant differences reflected in spatial working memory (Williams et al., 2006). These findings in congruence with this study support the idea for a mediating role for social memory, implying that firstly, it is different from objective memory for non- social stimuli. Furthermore, individuals with autism do not have the ability to differentiate between social and non- social stimuli, and hence do not discriminate between the stimuli with regard to reward value or encoding/ memory pathways, consequently resulting in social behavior deficits.

Though studies with regard to contextual recognition, memory or learning with respect to autism and social stimuli remain limited, studies have been conducted using models of similar neuropsychiatric disease like Fragile X syndrome. Baker and colleagues (2010) found impaired spatial learning and memory in Fmr1 KO mice bred onto an albino C57BL/6J background, but more interestingly these KO mice exhibited contextual memory deficits when conditioned with signaled and un-signaled shocks. Though not directly translatable to autism like social behavior deficits, such contextual memory deficits can be effectively modeled in MIA- mediated behavior deficit models to further support the idea that recognition and memory in the social context might develop typically to each aid with the neuro-specialization for the other. For example, intact social recognition ability would help in the encoding of social stimuli, making it more likely for this information to be stored. Conversely, impairments to social memory would still create behavioral deficits in tasks like

social recognition when measured both at PND30 and PND60, as Poly I:C offspring would not have a stored memory from PND30 and hence, would be unable to show a social preference. This implies that impairments to either or both social recognition or memory would be sufficient to cause autism like social behavior deficits.

Recognition scores were calculated for both treatment conditions and age groups as a measure of what percent of the total test time mice from each condition were spending preferentially in the chamber with the novel stranger mouse. A higher positive R score indicated preferential interactions in the novel stranger chamber and was used to determine whether MIA had impacted recognition of the novel and familiar stimuli. Poly I: C showed a significantly lower R score at PND30 than saline mice, indicating that they were not preferentially engaging with the novel mouse at this developmental age. Additionally, at PND60 the R scores for Poly I:C offspring are not significantly higher than saline offspring, suggesting an aversion to social stimuli through development. The trends with these scores further support chamber preference data, showing how Poly I: C offspring never achieve a social preference or intact recognition even at PND60, hence showing a developmental deficit in social recognition and memory. Conversely, confirmatory factor analysis studies have found co-occurrence of key clinical features like hyper sensory responsiveness and restricted/ repetitive interests to both conditions, suggesting that a similar

neurobiological mechanism mediates more global cognitive development, that evolve specific to either neurodevelopment condition (Boyd et al., 2010).

When considering the social cognitive development of the experimental mouse in this paradigm, it is also important to take into account any behavioral reciprocity by the stimuli this mouse is exposed to and how they might be impacted by inherent behavioral differences. Novel stranger stimuli mice were always age and sex matched to the experimental mouse, though stimulus mice included both saline and Poly I:C offspring, along with additional litters that were untreated and generated for the purpose of having stimulus mice. The previous treatments the novel stimulus mouse was exposed to was not controlled for with regard to the experimental mouse, and this could be considered a possible confound if mice exposed to saline have inherent behavior differences from untreated C57 mice. However, a study by Yang and colleagues (2012) to determine the effect of partner strain on sociability in low sociability exhibiting BTBR T+ tf/J versus highly social C57 BL/6J mice suggest that variations in partner preference do not alter sociability in the three chambered social approach task. This study found that typically developing C57 mice were highly social regardless of the strain of the novel mouse in the three- chambered social approach test. In the light of these findings, the strain of the novel stimulus mouse can be ignored as a potential confound to the results of this study. To address this confound, the experimental and stimulus mouse were matched on the basis of treatment. A very small number of the experimental group were not matched in a

similar way due to a lack of mice that fit the existing parameters i.e. age, sex, treatment, and additionally no previous exposure to the experimental mouse. Novel stranger stimuli mice were occasionally younger than the experimental mouse, and since experimental pairings were sex- matched, aggression and dominant behavior needs to be considered as potential confound. Additionally, the effect of age on social partner preference has not been investigated and could pose a potential confound to this study.

This experiment did not address if spatial learning and memory is related to social recognition ability. The behavioral testing method involved four phases; namely a central chamber habituation, complete apparatus habituation, social recognition task using familiar littermate and novel stranger mice and finally, a comparison between the stranger mouse from the previous round to a new stranger mouse. The last phase was conducted in order to determine the whether social memory deficits were short- term or long term in nature. According to species typical behavior, it was hypothesized that saline mice would spend more time with the second novel stranger mouse in the fourth round of testing, as it would be able to recognize the earlier novel mouse as familiar, and choose to engage with the novel social stimuli. Conversely, Poly I:C offspring would fail to differentiate between the novel stranger mouse in the fourth round, and resultantly show no interaction zone or chamber preference suggesting that these behavioral differences might be caused by deficits to social memory. Data from the last cohort did not support these hypotheses; Poly I: C offspring were found to be

spending more time in direct interaction with the novel stranger in comparison to the acquainted stranger mouse both at PND30 and PND60. Furthermore, Poly I: C mice also performed better at social recognition in comparison to their individual performance for zone preference at PND30, and spent more time in direct interaction with the novel stranger mouse. This highlights that Poly I: C might not be mediating social recognition and memory deficits at all, and results from the previous cohort might have been by random chance.

Future Directions

Though studies have not directly linked spatial learning and memory with social memory deficits in autism, Moretti et al. (2006) showed impaired spatial learning and memory, social memory and long- term potentiation in a transgenic mouse model of Rett Syndrome, which shares certain commonalities with autism with regard to social interaction and communication. Moretti and colleagues used tasks like the Morris Water Maze, contextual fear conditioning and three-chambered social recognition to identify hippocampus- dependent spatial memory, contextual fear memory and social memory were significantly impaired in male mice expressing a truncated allele of *Mecp2*, and X- linked methyl CpG binding protein that has been linked with Rett Syndrome. In the MeCp2 symptomatic mice, reduced post- synaptic density length in the CA1 areas of the hippocampus, paired with enhanced basal transmission in Schaffer- collateral synapses suggested heightened neurotransmitter release and impaired long term

potentiation. Additionally, these mice also showed impairments in long- term depression at the Schaffer synapses, suggesting that at the synaptic level, there is heightened neurotransmitter release in areas such as the hippocampus, paired with deficits in both long term potentiation and depression, creating an overall state of heightened synaptic activity that is not maintained or pruned, resulting in social memory deficits. It is important to note that social memory has not been studied at the cellular or transmission level, and similar experimental models can be adapted to specifically study MIA mediated social memory deficits. Measurements of long- term potentiation and depression are also indicators of learning and plasticity, and can be adapted to determine how MIA might impact neurospecialization specific to social recognition and memory.

Furthermore, dysfunctions in cortical GABA signaling linked to MeCp2 have also been implicated in autism- like stereotyped behavior (Chao et al, 2010). Additionally, animal models for Tuberous sclerosis complex (TSC), a disorder clinically characterized by high (25- 60%) prevalence of autism spectrum disorders and cognitive impairment have shown heightened glutamate activity linked translational differences at the synapse, implying a relationship between exaggerated protein synthesis that remains unregulated and the autistic brain (Kelleher & Bear, 2008). Genetic associations between autism and the maternal duplication of 15q11-q13 and resultant regulatory protein abnormalities have been specifically implicated within $GABA_A$ receptor subunit expression and binding have been found to be abnormally low in ASD patients, suggesting a lack of

inhibition in these areas that are responsible for memory (Belmonte et al, 2004). This seemingly tangential discussion of single- gene disorders that have a shared comorbidity with conditions like autism illustrates a gap in the investigation of environmental factors and how they mediate neurodevelopmental deficits. Social behavior deficit phenotypes have been described via multiple genetic models with a plethora of techniques, including behavioral assays, protein presence and localizations, transmission, spine density and electrophysiological studies. However, such experiments are yet to be established in environmental models, where such data could be used to support MIA- mediated social recognition and memory deficits that were only found using behavioral assays.

Another study implicating the role of Poly I: C mediated MIA in $GABA_A$ receptor expression by Nyffeler et al. (2006) showed heightened $GABA_A$ immunoreactivity in adult mice, suggesting that prenatal immune activation linked disturbances inflicted on neurodevelopment significantly alters limbic $GABA_A$ receptor expression, implicated in neurodevelopmental disorders like autism and schizophrenia. These findings from animal models of other conditions that are genetically linked with autism in congruence with the results of this study suggest that there might be a global impairment to the regulation of social recognition in neurodevelopmental disorders. A lack of inhibition that might be speculatively linked to deficits in $GABA_A$ transmission could impact preference for encoding, processing and retrieving social versus non- social stimuli. This indicates that the nature of social recognition and memory in deficits in disorders

like autism and schizophrenia are linked with a lack of inhibition, i.e. not only the inability of the brain to learn the difference between novel and familiar social stimuli, but also not be able to discriminate between these stimuli at a later time, indicating memory deficits. Speculatively, this suggests that MIA mediated social cognitive deficits could be related to a lack of inhibition, and resultantly, poor neurospecialization for social stimuli. This study supports the idea of MIA mediated social cognitive deficits, though the differences need to be analyzed at a structural and functional level within the brain to gain a deeper understanding.

Applications to the Field

The findings from the current study show that MIA mediates social recognition deficits in C57 mice as Poly I:C offspring do not show a social preference as juveniles, and neither as adults. MIA targets an immune- pathway targeting a specific subset of T- helper cells within the mother's immune system, namely Th1 cells that are activated in response to viral and bacterial pathogens. This study can be adapted to study social cognitive deficits that might be caused due to maternal immune insult mediated by another sub- population of T- helper cells, i.e. Th2 cells that have evolved to be exceedingly sensitive to environmental allergens.

Furthermore, as discussed earlier, there is a gap in research on the neurological basis of social memory, though evidence for the preference for social stimuli in both typically developing humans and mice suggests that that social stimuli might be encoded and neuro-specialized differently from non-social

stimuli. It is important to investigate why social memory would be different in a model for MIA- mediated neurodevelopmental deficits perhaps differently or in interaction with genetic models of the same deficits. These deficits themselves suggest that there are changes in the process of processing, encoding and retrieving social stimuli and animal behavior assays that are more sensitive to address these questions need to be developed. For example, since mice are olfaction dominant, an olfaction- linked conditioning task could be used to determine whether there is any difference in the way reward is learnt using social versus non social olfactory stimuli, like the urine of a novel mouse compared to almond essence respectively. Additionally, longitudinal behavioral testing within the same animal using object recognition, social approach and social recognition would help tease apart the development of social memory in interaction with social recognition using well characterized behavioral assays. Finally, all these models can be adapted to understand how MIA specifically impacts the development of social memory or social recognition or how MIA specifically impacts the learning and plasticity within the brain to create neurospecialization in favor of social interactions through neurodevelopment. Gaining a cellular and molecular understanding of this process using techniques like immunohistochemistry, analysis of spine densities, axon path-finding specific to certain brain regions during neurodevelopment, electrophysiology, synaptic signaling and pruning markers would be helpful in supplement to social cognitive deficits characterized by behavioral assays.

CONCLUSION

The following experiment was conducted in to determine how maternal immune activation impacts the development of social recognition and memory to model social behavior deficits that occur in neurodevelopmental disorders like Autism and Schizophrenia. Pregnant C57 dams were either treated with Poly I: C, a viral mimic that creates an elevated immune response, or saline. Offspring were behaviorally tested as juveniles and adults; it was found that Poly I: C offspring were unable to engage in novel social interactions as juveniles, and this deficit continued into adulthood, as they showed no chamber preference as adults either. Furthermore, this trend was not attributed to differences in locomotor performance or percent time spent exploring either chamber between Poly I: C and saline mice. These findings could not be replicated in the following cohort, data from which suggest that MIA does not cause social recognition and memory differences in Poly I: C offspring. The findings from this study highlight the need to firstly characterize how MIA creates a social behavior deficit at the behavioral, structural and functional level, and to develop behavioral tasks in the future that would be reliable in specifically addressing social recognition and memory and their implications in neurodevelopmental disorders.

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