Autism-relevant behavioral outcomes in an antigen-driven rat model of maternal autoantibody-related autism

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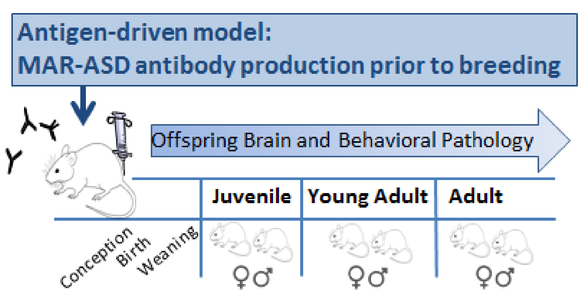
Introduction

- Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social development paired with repetitive behaviors.
- Immunoglobulin G (IgG) autoantibodies reactive to fetal brain proteins are present in 23% of mothers of children with ASD compared to less than 1% in mothers of typically developing (TD) children, raising the possibility of a maternal autoantibody related (MAR) form of ASD.
- The mechanisms through which MAR autoantibodies impact fetal neurodevelopment and subsequent behaviors in the offspring have yet to be elucidated.
- The laboratory rat provides an ideal preclinical model to further evaluate the effects of prenatal MAR antibody exposure on social development.
- Hypothesis: Rat dams that break tolerance and produce MAR autoantibodies will produce offspring that develop deficits in ASD related behaviors.

Methods

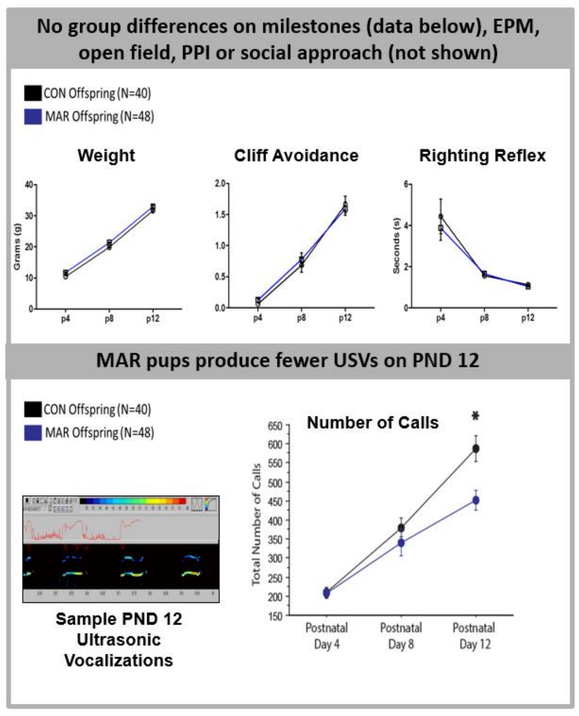
- Prior to breeding, female Sprague Dawley rats were randomly assigned to MAR (N=6) or CONTROL (N=6) treatment groups.
- Animals in the MAR treatment groups received a series of immunizations containing peptide epitope sequences of the 4 primary target proteins of MAR ASD (LDH-A, LDH-B, STIP1, and CRMP1) and adjuvant.
- Controls received PBS and adjuvant only.
- Following confirmation of autoantibody production to the salient epitope sequences, females were paired with male breeders to produce the experimental offspring of interest.



Offspring Behavioral Testing

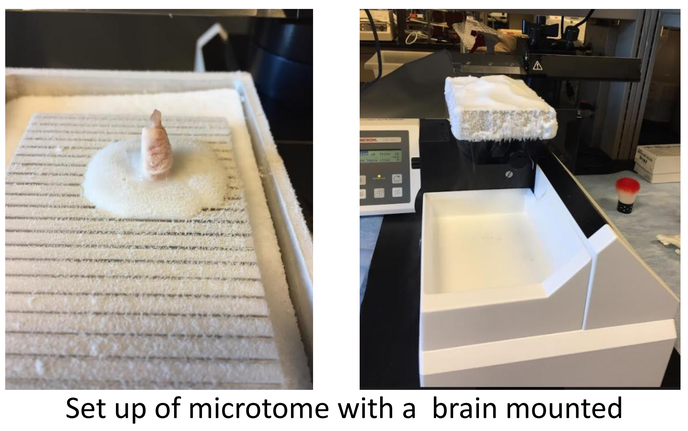
Litters from treatment and control groups were culled to four males and four females at post-natal day 2 (PND2) and underwent an evaluation of postnatal developmental milestones (measured at PND 4, 8, and 12). During the brief separation from the dam the number of isolation ultrasonic vocalizations (USVs) were quantified. Following weaning at PND 21, two male and two females from each litter participated in a behavioral test battery that began at PND 26 and concluded at PND 103. Testing assessed a range of behaviors including exploration, sensorimotor gating, anxiety, repetitive behaviors and sociability. Tests were conducted on separate days in the following order to reduce the influence of sequential testing: elevated plus maze, social approach, open field, pre-pulse inhibition (PPI), and social dyad.

Behavioral Test	Postnatal Day (PND)	Description	MAR vs CON
Developmental Milestones	4,8,12	Screen for developmental delays (motor, reflex etc.)	No differences
Isolation USVs	4,8,12	Quantify USVs in response to temporary separation	MAR<CON (PND12)
Elevated Plus Maze (EPM)	26 96	Anxiety related behaviors	No differences
Open Field	29 100	Exploratory locomotion	No differences
Pre-pulse Inhibition (PPI)	34 102	Sensory gating	No differences
3-Chamber Social Approach	28 98	Automated assessment of interest in a novel animal	No differences
Social Dyads	36 55 103	Fine grained analysis of 10min reciprocal social behavior with a novel partner	MAR<CON Social interaction MAR>CON Repetitive behaviors

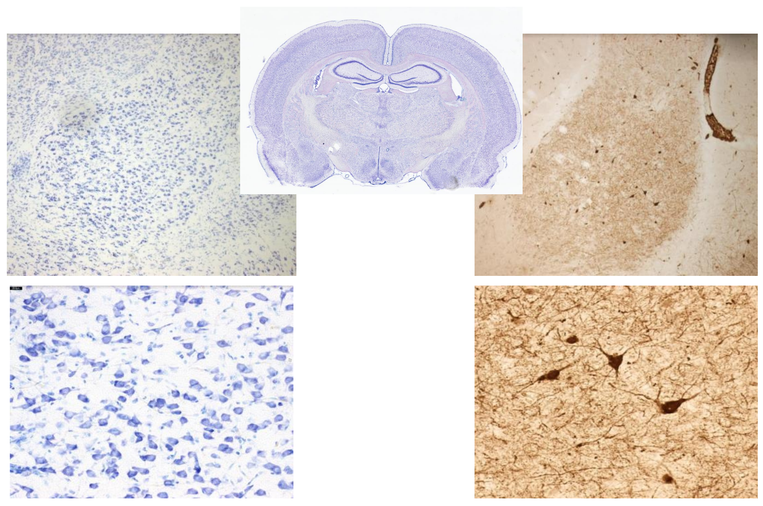


Amygdala Neuropathology Pilot Study Parvalbumin Expression Preliminary Data

- Sections of right hemisphere from a separate cohort were fixed then sliced with a microtome
- Developed protocol to conduct Nissl Stain to specify the basolateral nucleus in the rat amygdala
- Developed protocol to conduct a Parvalbumin immunohistochemistry stain to further quantify the expression of PV+ cells
- Up to date: currently we have finished Nissl staining and PV staining, next steps will be to pilot a protocol for a quantitative analysis



Set up of microtome with a brain mounted

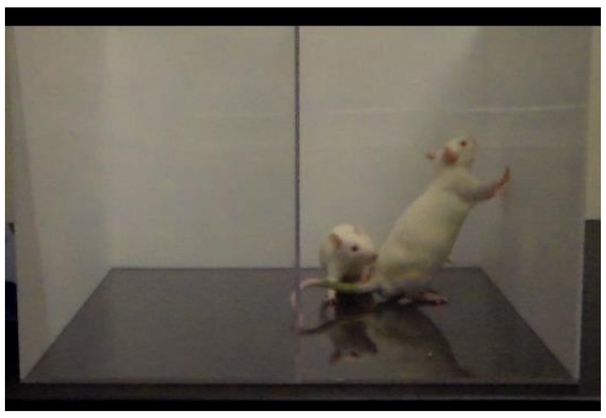


Nissl Stain PV Stain

Future Directions

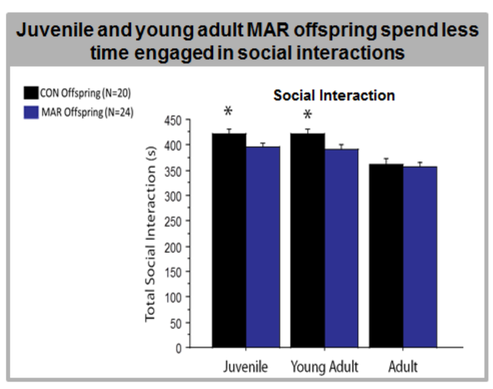
- Detailed analysis of reciprocal social interactions will be carried out to further characterize the social deficits exhibited by the MAR offspring:
  1). Two Chamber Social Dyad
  2). Fine grained analysis of "Nape Attacks"
- Quantitative analysis of PV+ cell numbers in the basolateral amygdala using stereology and integrating *in vivo* neuroimaging to provide insight into underlying neuropathology associated with prenatal exposure to autism-associated maternal antibodies.

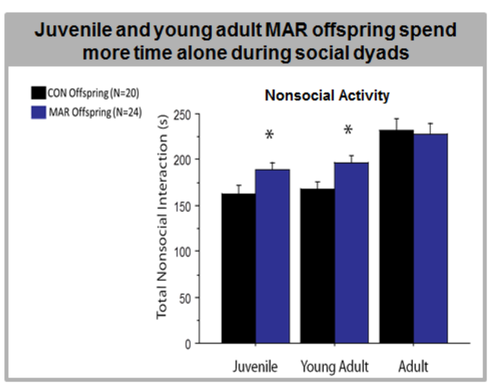
Two Chamber Social Dyad

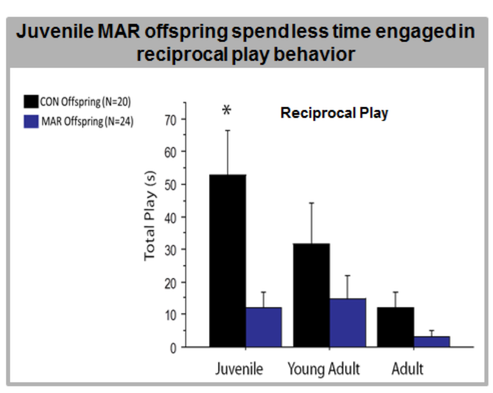


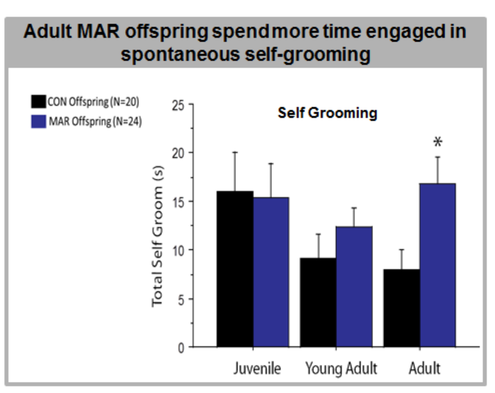
The treated experimental rat and its partner stimulus rat are put in a chamber with a divider in the middle. Social interactions are recorded then scored using an ethogram and Observer Software at PND 42 and 75. One finding of interest was that treated animals withdrew from socialization more frequently.

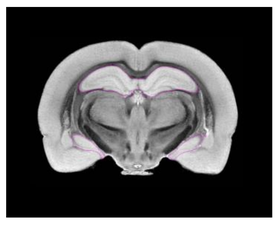
ASD Relevant Behavioral Alterations











MRI of rat brain

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1). E. Edmiston et al., Autoimmunity, Autoantibodies, and Autism Spectrum Disorder. Biological Psychiatry 81, 383 (Mar 01, 2017).

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