Proposal Example 3

Title and Hypothesis (1000 characters maximum):

Brain and behavior changes in rodent offspring prenatally exposed to autism associated maternal antibodies

MAR treated offspring will produce and receive fewer bouts of play behavior initiation than control offspring. Neurons of MAR treated offspring will be larger and/or exhibit greater dendritic complexity than neurons of controls.

Specific Aims (2000 characters maximum):

Numerous studies have now demonstrated that maternal autoantibodies to proteins highly expressed in the developing brain are both excellent biomarkers of ASD risk and pathologically significant [1-3]. Early research by Dr. Judy Van de Water and colleagues identified two bands at 37 kDa and 73 kDa, that were significantly more common among mothers with autistic children and associated with aberrant brain development [4-6]. Specific combinations of these autoantibodies to fetal brain proteins were predictive for autism risk [7], suggesting the presence of a distinct, maternal antibody related (MAR) subset of ASD cases. An essential next step in this promising line of research is to determine the pathogenic mechanism by which these antibodies lead to the behaviors observed in children with ASD. Preclinical research using animal models provides a platform to explore the effects of MAR autoantibodies on brain and behavior development. Rodents and nonhuman primates prenatally exposed to MAR autoantibodies via passive transfer show alterations in neurodevelopment that parallel features of human autism [8-12]. For my STAR research project I propose to further characterize brain and behavior development in rat offspring that have been ***continuously*** exposed to MAR antibodies using a novel antigen-driven model.

Specific Aim 1: Carry out a fine-grained analysis of reciprocal social interactions in juvenile MAR and controls offspring rats.

Specific Aim 2: Define the neuropathological consequences of prenatal exposure to the epitope-specific maternal autoantibodies in offspring by quantifying the size of neurons in the dorsolateral prefrontal cortex (DLPFC) in MAR treated offspring.

Project Plan Significance (500 characters maximum):

Our proposed project plan will address the critical need to understand the etiology of ASD by determining the pathological significance of maternal autoantibodies in ASD and their effects in brain and behavioral development. This highly innovated and translational model will serve as a preclinical platform to advance precision medicine efforts in developing highly tailored therapeutics that address the underlying biology of ASD.

Innovation (500 characters maximum):

The proposed research utilizes a novel, antigen-driven animal model to explore the effects of continuous prenatal exposure to MAR autoantibodies. The laboratory rat provides a unique opportunity to integrate fine-grained analysis of reciprocal social behavior with a preliminary assessment of underlying brain pathology, thus maximizing the translational potential of this novel animal model.

Approach. Must include Rationale and Methods, Potential Problems and Alternatives, Experimental Rigor (statistics, validation of reagents, sample size, etc.). 8000 characters maximum.

Rationale:
Autism spectrum disorders (ASD) are a set of neurodevelopmental disorders classified by core impairments in social interactions and communication accompanied by the presence of repetitive and stereotyped behaviors [13, 14]. Despite increasing prevalence rates and awareness of the disorder, the causes for idiopathic ASD are unknown. Although genetic factors are thought to have an important role in the etiology of ASD, recent evidence suggests that environmental influences during gestation or early postnatal periods also contribute to development of ASD [15]. Immune system dysregulation and reactive maternal autoantibodies towards fetal brain proteins are potential non-genetic factors that have been identified among mothers of children with ASD [1-3]. Animal models such as rodent and nonhuman primates prenatally exposed to MAR autoantibodies have been used to explore effects of MAR autoantibodies on brain and behavior development showing alterations in neurodevelopment that parallel features of human autism [8-12]. This project will utilize a novel antigen-driven model to explore, for the first time, effects of continuous prenatal exposure to MAR antibodies.

Preliminary data:
Passive transfer of MAR antibodies via single injection to the dam at mid- gestation or in utero intraventricular injection to the fetal brain in mouse and rhesus monkey (Macac mulatta) models produce offspring with ASD-relevant changes in brain pathology and behavioral development that parallel features of human ASD symptomatology [9-11] [8, 12]. All animal models to date have utilized passive transfer techniques, in which pregnant animals were injected during mid-gestation with human IgG isolated either from mothers of typically developing children or from mothers of children with ASD. However, these models do not reflect a constant exposure throughout gestation, as would be the case in the clinical setting. Dr. Melissa Bauman, in collaboration with Dr. Judy Van de Water, developed an antigen-driven animal model of MAR risk for ASD using rat embryos continuously exposed to maternal antibodies throughout gestation to directly assess the pathologic significance of prenatal exposure to epitope-specific autoantibodies in generating ASD-relevant behaviors in offspring. Rat offspring born to these dams demonstrate altercations in communication, social development and repetitive behaviors that closely parallel human ASD symptoms. This STAR project will carry out a more detailed evaluation of these social impairments and a first assessment of underlying neuropathology of MAR treated offspring.

Aim 1 Methods:
Archived videos of social interactions comparing MAR and control offspring will be used as preliminary evaluation of these videos showed that MAR rats spend less time engaged in social behaviors throughout development, particularly juvenile play behavior. A fine-grained evaluation of these archived videos will quantify the exchange of species-typical reciprocal social interactions to determine if the decrease in social interaction is driven by (i) failure of MAR treated offspring to initiate interactions or (ii) failure of the untreated stimulus rat to respond to MAR offspring social interactions (previously observed in the nonhuman primate model) [8]. Archived videos from both MAR (N=24) and control (N=24) interactions with novel partners at juvenile (postnatal day, PND 36) will be used. After establishing laboratory standards of inter- and intra-rater reliability, videos will be scored using Observer XT12 software (Observer Version XT12, Noldus information Technology, the Netherlands). Focal observations will be used to quantify frequency of play behaviors initiated and received by the focal rat using a behavioral ethogram described in our previous work [16]. Coders will remain blind to the experiential condition of the animals throughout the study.

Aim 2 Methods:
We will examine how maternal autoantibody exposure impacts the brain size and weight in the mature prefrontal cortex in MAR rat relative to controls. Although there are many brain regions more directly associated with social behavior (i.e., amygdala), we propose to focus on neuropathology in the dorsolateral prefrontal cortex (DLPFC), which was implicated in clinical studies from MAR exposed children [6] and our previous preclinical models [8, 10]. Brains will be extracted and processed in the spring of 2018 and available for the STAR project in summer 2018. The DLPFC will be dissected and post-fixed for stereology (left hemisphere independently evaluated by Bauman Laboratory) and Golgi (right hemisphere for STAR project) following our established DLPFC neuroanatomical protocol using Stereoinvestigator and Neurolucida software (MBF Bioscience) [17,18]; remaining tissue will be archived for future studies. Golgi- Cox protocols for rodent tissue preparations to obtain high quality staining will also be used. We will randomly select, trace, and 3D reconstruct ten pyramidal neurons per case to measure soma area, total dendritic length, mean segment length, number of branches, and number/density of spines.

Potential Problems and Alternatives:
The Bauman Laboratory has extensive experience with the behavioral and histological technique proposed and does not anticipate any issues with the proposed research. Experiments have been designed to accommodate the 10 weeks STAR schedule: (i) The first week dedicated to training, lab safety, and reliability assessments, (ii) Only juvenile social videos will be coded (N=48 videos @10min= 5-6 videos/week for 8 weeks), (iii) Golgi reconstruction is particularly labor-intensive and will require additional undergraduate student support to complete N=10 neurons per brain (N=320 total neurons) or 40 neurons/week for 8 weeks. I will receive training and supervision from Dr. Bauman on behavioral data coding and from recruited collaborator, Dr. Cynthia

Schumann, for histological analysis. Dr. Van de Water will also help consult on the experiments and interpretation of the MAR data with Drs. Bauman and Schumann. We will utilize the Biostatistics Core of the UC Davis Intellectual and Developmental Disabilities Research Center (IDDRC) to carry out all statistical analyses.

Experimental Rigor:
Neuron number, size, and dendritic morphology will be compared between groups using the appropriate statistical model determined by the statistician on our team (see power calculations below). Based on previous studies in mice prenatally exposed to ASD-specific antibodies [10], we anticipate that MAR treated rats will show increased size of prefrontal cortical neurons and an increase in the extent of dendritic complexity. Preliminary power calculations carried out using Stplan v4.5. With a total of 32 brains (group 1 including 16 rats prenatally exposed to MAR antibodies; group 2 including 16 controls), a power of 83% will detect a difference of 1.5 standard deviations of histological outcomes between the two groups by using the two-sided t-test with the equal standard deviation assumption at a significance level of 5%.

Conclusion:
The proposed studies have been designed to maximize my research experience during the 10 week STAR program and provide meaningful contributions to Dr. Bauman's program of research. In addition, I will be provided with weekly targeted training opportunities with other laboratories including to enrich my research experience (see 10 week training plan outline).