Bharat Biswal

PROJECT SUMMARY/ ABSTRACT
TITLE: Altered Brain Connectivity in Autistic Patients

Autism encompasses a broad range of brain disorders that, by conservative estimates, affects 1 in 500 children. Autism-Spectrum Disorders (ASDs) include Asperger's Syndrome, Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), Rett Syndrome (which affects only girls) and Autism. Autism Spectrum Disorders impair three main areas of human development: speech, communication and social interaction. These impairments may range from mild to severe.

Recently, many studies of Autistic individuals have focused on response to and processing of human faces, which are difficult tasks for this group of individuals. In one study, Autistic subjects who viewed photographs of faces had lower activations compared to control subjects in the inferior left prefrontal area, which is important for verbal processing and memory and the right posterior temporal area, a region implicated in mind processing (Koshino et al., 2007). This study also found that the Autistic subjects showed activation in a different location in the fusiform area than did the control participants. While the above proposed fMRI studies compare amplitude changes of the blood oxygenation level dependent (BOLD) signal between a stimulus and a control/baseline condition, these do not account for the brain's network organization. It is well known that different anatomical regions in the brain cooperate and interact in order to perform a motor or cognitive task. And in these studies, it is very important to quantify how brain networks are different between healthy controls and Autistic patients. To address this, a promising methodology of fMRI based functional connectivity (fcMRI) is available. The underlying principle of resting state fcMRI is to probe spatio-temporal synchronizations of spontaneous low-frequency BOLD fluctuations (LFBF). These slow oscillatory BOLD signal changes were first observed by PI (Biswal et al, 1995). There is a growing interest in studying disease related alterations in fc over a range of neurodevelopment and neurodegenerative diseases such as Multiple Sclerosis (Lowe et al, 2002), Alzheimer's disease (Li et al, 2002) and a bunch of psychiatric diseases such as – Schizophrenia (Garrity et al, 2007; Liang et al, 2006), depression (Anand et al, 2005; Greicius et al, 2007), attention deficit-hyperactivity disorder (Tian et al, 2006; Castellanos et al, 2008) and autism (Kennedy et al, 2008; Cherkassky et al, 2006). Consistent reduction and abnormality in fcMRI have been reported in some studies based on either selective region of interest or whole brain analysis. One of the goals of this proposal is **to compare the alterations in resting state functional connectivity between Autistic and healthy controls.** The methodology and the techniques developed in this proposal can be used by other investigators to isolate and measure neurological disorders. The completion of this proposal would lead to: **1. greater understanding of brain function in Autistic patients**; and **2. lead to effect of various clinical interventions and treatment on the brain connectivity in Autistics.**

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **D'Arcangelo, Gabriella**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Reelin abnormalities in synaptogenesis and ASD**

Please provide a one sentence description of your project
In this proposal we will investigate the contribution of the Reelin pathway, a molecular signaling cascade that is known to regulate many aspects of brain development, to the risk of ASD.

Description (Do not exceed space provided on this page. Type in single spaced format.)
Cognitive and behavioral disorders such as autism are thought to result from defects in brain cell connectivity and function that occur during development. Proper brain function requires that cellular components of the cerebral cortex, the cortical neurons, develop anatomical structures called dendritic spines. These structures establish contacts (the synapses) with processes called axon terminals coming from other neurons. The axon terminals release neurotransmitters that are received by the dendritic spines and thereby transmit the electrical inputs that underlie brain activity. The molecular mechanisms that control spine development, and thus the formation of cortical neuron circuitry, are not well understood. Genetic studies in human families reveled that several proteins that are involved in the function of the synapse are disrupted in patients with autism. My laboratory discovered that two proteins called Reelin and Dab1 form a signaling cascade that critically regulates brain development, and specifically promotes the formation of dendritic spines. In the first part of this proposal we will study how these proteins affect the development of spines in the cerebral cortex of mutant mice. We will also examine whether autism-associated synaptic proteins are abnormal in mutant mice deficient in Reelin activity to determine whether these mice are viable models for the disease. In the second part of this proposal we will investigate the activity of Reelin in blood-derived human cell lines obtained from patients with ASD and control subjects to determine whether there is a deficit in Reelin signaling in the patients. These studies will clarify the link between Reelin activity and autism and lay the foundation for future translational studies aimed at developing new therapeutical approaches for this devastating disease.

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **DICICCO-BLOOM, EMANUEL M.**

LAY ABSTRACT OF RESEARCH PROJECT

En2 Regulates Forebrain Monoamines and Behavior

Please provide a one sentence description of your project:
Define the role of the hindbrain patterning gene En2 in development of forebrain monoamine neurotransmitters, regional brain structure and autism related behaviors.

Autism Spectrum Disorder (ASD) is a pervasive developmental disorder characterized by abnormalities in social communication, language, and restricted and repetitive interests/behaviors. Genetic factors play a major role in disease causation. Importantly, many individuals with ASD, up to 40%, have increases in the blood of a neurotransmitter called serotonin, or 5HT, suggesting that its development or regulation are altered, and may contribute to the symptoms. We recently found that a genetic difference in a gene (Engrailed 2, EN2) that regulates development of the hindbrain (in the back of the skull) affects the normal growth of neurons that make serotonin and other related monoamine-containing neurons, such as norpinephrine. These neurons that originate in the hindbrain project directly to the forebrain and affect mood, aggression, attention, social behavior and more. We now plan to investigate the effects of EN2 by looking at mice in which EN2 is deleted, so-called knock out mice. We will measure the levels of these monoamine transmitters in both the forebrain and hindbrain. Then we will analyze their nerve fibers to understand how their development is affected when EN2 is missing, and correlate this to MRI brain imaging to see how brain region volumes are affected, as observed in people with ASD. Finally, we will perform specific behavioral tests that measure changes we predict to occur because of the abnormalities in monoamine transmitters. We have already found that the monoamine levels in the forebrain are reduced, that these animals have fewer axonal processes in the cortex and hippocampus, and they display a "depressed-like" behavioral state, suggesting that some of the ASD phenotype is modeled by these knock out mice. Because we know what has changed in these neurotransmitter systems, we may have the opportunity to correct these behavioral deficits, and develop new approaches to people with the disorder. Significantly, risperidone, the only FDA approved drug for ASD, directly regulates these same monoamine systems.

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Bonnie Lynne Firestein, Ph.D.**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Regulation of Synaptogenesis by Cypin and Neuroligin-1**

Please provide a one sentence description of your project
This project will identify how the proteins cypin and neuroligin-1, which has been linked to autism, regulate how neurons communicate.

In order for the brain to function normally, there must be a balance between positive, or excitatory, and negative, or inhibitory, information that is processed by neurons. In autism, this balance is disturbed. The proposed experiments will address how two proteins, called cypin and neuroligin-1, regulate regions where excitatory information comes into a neuron. These regions are called synapses. Cypin breaks down compounds called purines, which are altered in patients with autism. Neuroligin-1 is encoded by a gene that has been implicated in autism. The project will use cultured neurons grown in dishes to ask how cypin and neuroligin-1 regulate the number of excitatory synapses. Using DNA technology, either cypin or neuroligin-1 will be increased or decreased in the neurons. The amount of excitatory synapses that result will be determined. Using biochemical techniques, it will be determined how cypin and neuroligin-1 change excitatory synapses. The proposal will address whether cypin and neuroligin-1 change the degradation or the production of other proteins that act to build the synapses. Once we understand how excitatory synapses may change in autism, we will be able to target drugs to fix abnormal development of these synapses. Furthermore, by understanding how cypin and neuroligin-1 act in the neuron, we may be able to identify other proteins, and hence genes, that may be involved in autism. We can use these genes or proteins to then screen for autism. Since New Jersey has a high population of children with autism (approximately 1 in 84 eight year olds), understanding how the brain develops and what may go wrong in autism will help us to develop therapies for these children whose brains are still developing.

Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Janvier, Yvette**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Autism outreach/screening in under-served areas**

Please provide a one sentence description of your project **This study would evaluate the effectiveness of community-based autism outreach and education to under-served populations**

Research suggests that people from low income or ethnic minority groups may have difficulty accessing services for early detection and treatment of autism. The purpose of this study is to increase the early identification of autism spectrum disorders (ASD) in traditionally underserved young, low income, minority children and help these children to get early intervention services. We will train healthcare providers in five target communities in New Jersey with large low income and minority populations to conduct developmental screening to children in their practices. In partnership with community leaders, culturally-sensitive outreach materials will be created to cover child development, behavioral signs of possible developmental delay and resources regarding access to child health services. These materials will be used to provide autism education workshops in easily accessible locations throughout these five communities to large groups of parents/guardians, caregivers, early intervention staff, and other community members. Autism developmental screening clinics will be held in each of the five communities in easily accessible locations for children referred by community healthcare or education providers, parents/guardians, or early intervention staff. Those children in whom either generalized developmental delays or symptoms of ASD are found will be referred to appropriate intervention programs. They also will be linked back to their community pediatrician or health clinic for follow up. Additional resources and referrals for hearing, speech/language evaluations or other services will be made as needed. Through the autism developmental screening clinics, we expect to screen 1000 young children in these five target communities over the two years of the grant. We expect to improve access to early diagnosis and intervention for a large number of underserved children who traditionally would not have been identified until after they entered elementary school, ultimately benefitting the children, their families, and the community as a whole, improving functional outcome and reducing ultimate lifelong cost for specialized services

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Jonakait, G. Miller**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Mediators of Maternal Inflammation**

Please provide a one sentence description of your project
We will investigate the placental T cell repertoire and fetal brain microglia for their possible roles in mediating maternal inflammation during fetal development.

Mounting evidence suggests that prenatal exposure to infectious agents with associated maternal and/or fetal inflammation may combine with a genetic predisposition to produce autism spectrum disorders. The mechanism(s) that could translate maternal inflammation into a neurodevelopmental disorder (and, often, an immunological disorder as well) remain vague. Our proposal investigates two possible sites that could serve as communication pathways from mother to fetus: **the placenta** and **fetal microglia**. A major role of the placenta is to protect the fetus from immunological rejection by the mother. This is accomplished in part by a newly-identified T cell type, called regulatory T cells (Tregs). The proper balance of Tregs to other T helper cells is necessary for normal fetal development. We will investigate this balance following maternal inflammation induced by a synthetic compound (poly[I:C]), that mimics a viral infection, or by the small molecule interleukin-6 (IL-6), shown recently to be an essential mediator of maternal inflammation in producing animal behaviors akin to those seen in autistic children. Blood-producing cells arising in the placenta move to the fetal liver during midgestation in the mouse. Arising from the fetal liver, brain microglia – the intrinsic immune active cells of the brain -- begin to colonize the brain soon after. Thus, immunological status in the placenta may have consequences for microglial status. We have recently shown directly that fetal microglia become inflamed following maternal inflammation. Microglial inflammation during embryonic brain development can skew important developmental events. Microglia from brains of fetuses from dams treated with and without poly(I:C) or IL-6 will be isolated and analyzed to determine the molecules that they secrete. The intimate association of microglia with an important signaling molecule, reelin (implicated in autism) prompts also a closer analysis of reelin changes that may occur following microglial inflammation.

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Lewis, Michael**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Self Representation and Brain Development in Autism**

Please provide a one sentence description of your project **The aim of this project is to study the relations between white matter development in brain and the developmental delays in acquiring self representation in children with autism spectrum disorder.**

Children with autism have difficulties with social relations. This is due in part to their delays in developing a mental representation of themselves. Most typically developing children show by age 2 the ability to recognize themselves in a mirror, to use personal pronouns correctly, such as "I" and "me", and to involve others in pretend play. These patterns of development of self representation are delayed in children with autism. They have delays in the development of the mirror recognition and in using pronouns to describe themselves. In addition, they show pretend play that involves only themselves rather than including others. Studies of the brain of typical children show that the ability to have self representation is related to the development of specific areas of the brain. These same regions are believed to be underdeveloped in children with autism. As a result of the delay in brain maturation, the child is delayed in experiencing self representation. This delay, in turn interferes with the abilities to interact with others, to show empathy, and to share. We plan to conduct studies of the images of the brain using high resolution techniques that have been used by the National Institutes of Health (NIH) to study hundreds of infants and children across the country. We plan to compare the images of the brains of young children with autism to the normative standards available from the NIH. In addition we plan to study the self representation abilities of the same children. In that way, we can relate the brain images to the self representation behaviors and identify the brain regions that are involved in the delays in the development of self representation that are shown by children with autism. When we have identified the brain regions involved in autism, we would be in a position to identify activities that may help to accelerate the maturation of the affected brain regions. This would require early interventions. It is possible that early intervention with activities that may accelerate brain maturation would also reduce the delays in the child's self representation.

Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Millonig, James H**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Genetic and epigenetic ENGRAILED 2 ASD risk factors**

Please provide a one sentence description of your project
The goal of our research project is to identify and characterize genetic and non-genetic factors that increase risk for ASD.

Description (Do not exceed space provided on this page. Type in single spaced format.)

Risk to Autism Spectrum Disorder is likely a combination of genetic susceptibility and non-genetic environmental factors. Our previous human genetic analysis identified the *ENGRAILED 2* (*EN2*) gene, an important regulator of CNS development, as a susceptibility locus. These studies demonstrated certain *EN2* genetic variants are inherited more often in individuals with ASD than unaffected siblings. *In vitro* molecular
analysis determined these genetic variants are functional, increasing gene expression levels. My lab has generated mouse models for these functional genetic variants. Preliminary *in vivo* analysis substantiates our *in vitro* studies, demonstrating the ASD associated genetic variants result in increased expression. Environmental factors can also influence gene expression levels. These factors can regulate gene expression through the differential methylation of cytosine nucleotides in DNA regions called CpG islands. Interestingly, numerous CpG islands are present in the *EN2* gene and preliminary data indicates these cytosines are differentially methylated. These data led to the following hypothesis: both genetic variants and non-genetic factors can increase ASD risk by elevating *EN2* levels. This hypothesis will be tested in the following two aims. Aim1: the functional effect of the ASD associated *EN2* genetic variants throughout CNS development will be investigated in our mouse models. Aim 2: to investigate if differential methylation is correlated with increased expression, *EN2* methylation and gene expression levels will be determined both in cell culture and in human post-mortem samples. These studies will determine whether both genetic and non-genetic factors increase ASD risk by elevating *EN2* expression. Future studies will investigate whether exposure to environmental factors during CNS development can worsen or improve the effect of the *EN2* genetic variants. This information will be essential for the development of new therapies and preventions for ASD.

New Jersey Governor's Council for Medical Research and Treatment of Autism
GRANT APPLICATION, Continued

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: **Shiffrar, Maggie**

LAY ABSTRACT OF RESEARCH PROJECT

Project Title (Do not exceed 52 spaces) **Perceptual-motor anticipation in individuals with ASD**

Please provide a one sentence description of your project **A series of experiments will determine whether individuals with ASD, like typicals, have visual and motor systems that spontaneously anticipate future events.**

Scientists, parents of and people with Autism Spectrum Disorder (ASD) have long known that ASD significantly limits social abilities. What remains to be established is the underlying causes of these social limitations. In the real world, social ability depends upon an individual's perceptual and motor skills; that is, whether one can rapidly and accurately perceive other peoples' actions and respond accordingly. Several scientists have started to consider whether perceptual and/or motor problems might ultimately cause the social difficulties associated with ASD. In typical individuals, perception and motor behavior are inherently predictive. For example, when trying to catch a ball, typical visual and motor systems calculate where the ball is going rather than where the ball is presently located. Anyone lacking such spontaneous anticipations would necessarily be trapped in an erratic and unpredictably changing world. There is reason to suspect that autism significantly reduces the visual and motor systems' ability to anticipate or predict future events. To discover whether people with ASD suffer from significant deficits in the anticipatory components of their visual-motor experiences, an outstanding group of researchers in the visual and motor sciences and clinicians specialized in the diagnosis and treatment of ASD propose to pool their expertise and conduct a systematic series of experiments. These studies will take advantage of recent technological advances in the non-obtrusive measurement of eye and limb movements by using specialized equipment already available at Rutgers, The State University of New Jersey. In the proposed studies, young adults with ASD and typical controls will watch and sometimes touch carefully controlled computer displays while their eye and limb movements are tracked. The computer displays will depict simple moving objects and the predictability of the movements of those objects will be systematically manipulated. Measurements of when and where participants look and how they move while viewing these displays will establish the predictive content of their perceptions and actions. Comparisons of these measurements between participants with ASD and typicals will determine whether, and if so, what type of the expectations are spontaneously generated by the visual and motor systems of people with ASD. The discovery of compromised visual-motor predictions could overthrow several dominant theories of the sources of dysfunction in ASD. It would also revolutionize current ASD treatments that tend to focus on the amelioration of social and cognitive deficits rather than on the perceptual and motion inputs into those higher-level behaviors.

Gleb Shumyatsky

Title: *Amygdala-enriched genes in behaviors related to ASD*

Summary sentence: This proposal will test which social ASD-related behavioral abnormalities are affected by amygdala-enriched genes and whether these behavioral deficits can be reversed.

Lay abstract:
The amygdala is critically involved in emotional responses, and behavioral social abnormalities characteristic to ASD. However, the molecular mechanisms governing these processes are unknown. To this end, behaviors related to ASD in several transgenic mouse lines generated based on the genes highly concentrated (enriched) in the amygdala will be examined. We will test the hypothesis whether perturbations in amygdala-enriched genes are critical for social and emotional states related to the ASD behavioral abnormalities. First, the three core ASD features, the abnormalities in social behavior, communication, and inflexibility/repetitive behaviors, will be probed as being controlled by amygdala-enriched genes.. Several knockout lines for amygdala-enriched genes will be used. Knocking out these amygdala-enriched genes should affect ASD-related behaviors and their stress sensitivity, demonstrating the role of local gene expression in the amygdala in ASD-related phenotypes. Second, a critical period during development for ASD-related behaviors will be studied and whether the behavioral deficits can be reversed in the adult. This will be analyzed using recently generated transgenic system in mice, allowing turning transgenes on and off. It will be examined at what developmental stage turning off abnormal gene expression in the amygdala-associated neuronal circuitry will reverse the behavioral abnormalities. It is expected to find a critical window in development when by turning the gene on and off we can control behavioral abnormalities. If the hypothesis is correct, future treatments of ASD with the focus on amygdala gene expression can be envisioned. Importantly, our new transgenic line is the first to restrict gene expression specifically to the amygdala neural circuitry and thus holds great promise for transgenic research of normal brain function as well as its pathology.

Harvey R. Weiss, Ph.D.

Autism and control of cerebral metabolism

Lay Abstract

One sentence description: This project will attempt to determine the changes in excitatory and inhibitory neurotransmitter system input in the control of cerebral metabolism in an animal model of autism and whether these defects can be corrected.

Autism can lead to severe difficulties in social interaction and communication as well as repetitive behaviors. Both the causes and treatments for this problem are not well characterized. There have been suggestions that the problems associated with autism are related to local regional cerebral imbalances between the excitatory and inhibitory neurotransmitter systems. We wish to study the effects of excitatory and inhibitory neurotransmitters on brain metabolism in regions associated with autism in an animal model. However, no good experimental models of this disease exist. Therefore, we will rely on the very strong association between tuberous sclerosis and autism and use a good tuberous sclerosis model (the Eker rat). Our preliminary data indicate dramatic changes in cerebral oxygen consumption and how consumption is controlled in appropriate brain regions of this model. These changes occur in very young animals. We think that there are major differences in how excitatory and inhibitory neurotransmitter systems control brain metabolism in those regions most affected by autism. We will, therefore, measure regional cerebral oxygen consumption and determine how activation or inhibition by these neurotransmitter systems affect regional cerebral metabolism. We will also examine changes in receptor density. Comparisons will be made between our model of autism and control animals. Our preliminary data indicate major differences in several of these systems. The experiments proposed in this application will allow us to determine which receptors and neurotransmitter systems are most affected in this model of early autism. It is also quite possible that multiple systems are involved. Once we know which receptors and systems are most affected in our model compared to controls, we will give chronic treatments of either antagonists or agonists. We may have to affect multiple neurotransmitter systems. Changes in regional brain metabolism will be determined. It is our hope that we can restore the way the brain controls its local metabolism toward normal. This could help to reduce the severity of the symptoms found in autism and autism spectrum disorders. We feel that these experiments will form a basis to decide on a rational pharmacological approach to the treatment of autism.