



**NJ Governor's Council for Medical Research and
Treatment of Autism**

Proceedings: Scientific Meeting

Basic and Clinical Sciences Research Grantees

National Conference Center at the Holiday Inn

East Windsor, New Jersey

March 23, 2012

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www.nj.gov/health/autism/

Purpose for the Meeting

The goal of all of The Governor's Council for Medical Research and Treatment of Autism (Council) is focused on helping Autism Spectrum Disorders (ASD) affected individuals and their families, and the healthcare systems serving them, to address their needs. Research in the basic and clinical sciences serve as the foundation and first step in discovering best practices in the early identification and treatment of ASD.

This meeting provided a unique opportunity for research colleagues to share information and set the stage for possible future collaborations. The intent of the Council is to raise the level of awareness among stakeholders of the impact of New Jersey autism research while positioning grantees to compete for federal funding. This is a new initiative by the Council and the start of what we hope to ultimately be a statewide program to bring all autism researchers together annually for a summit to present and hear about the "***State of the State in Autism Research in New Jersey***". *

**The changes in the higher education infrastructure in New Jersey as a result of the Rutgers-University of Medicine and Dentistry of New Jersey (UMDNJ) merger, effective July 1, 2013, are not reflected in this report.*

**NJ Governor’s Council for Medical Research and Treatment of Autism
March 23, 2012 Scientific Meeting of Research Grantees**

Table of Contents

	Page
Autism Spectrum Disorders (ASD)	4
The Governor’s Council	4
Council Membership	5
Scientific Meeting Agenda	6
Opening Remarks	7
Martin T. Zanna M.D., MPH, Acting Executive Director, Governor’s Council for Medical Research and Treatment of Autism	
Caroline Eggerding, M.D., Chairperson, Governor’s Council for Medical Research and Treatment of Autism	
Welcome	8
B. Madeleine Goldfarb, MA Member, Governor’s Council for Medical Research and Treatment of Autism	
Autism Research: Strengths and Needs in New Jersey	10
Dorothy Gaboda, M.S.W., Ph.D., Rutgers Center for State Health Policy	
Report on Autism Council Activities	13
Martin T. Zanna M.D., MPH, Acting Executive Director, Governor’s Council for Medical Research and Treatment of Autism	
Interdisciplinary Discussion Summation and Recommendations	17
<i>Michael Lewis, Ph.D., Institute for the Study of Child Development, UMDNJ-RWJMS</i>	
<i>Emanuel DiCicco-Bloom, M.D., Dept. Neuroscience & Cell Biology/Pediatrics, UMDNJ-RWJMS</i>	
<i>Dennis Carmody, Ph.D., Institute for the Study of Child Development, UMDNJ-RWJMS</i>	
<i>Yvette Janvier, M.D., Children’s Specialized Hospital</i>	
Funding Initiatives	25
In Memoriam to Steven Zalcman, Ph.D.	30
Contact Information	34
Appendix: List of Participants, Evaluation of Meeting and Research Reports	36

Autism Spectrum Disorders (ASD)

Autism Spectrum Disorders (ASD) are complex neurodevelopmental disorders with early childhood onset. The prevalence of ASD may be increasing, and ASD is more common than previously thought. These disorders, for which there is presently no cure and only limited treatments, generally have lifelong effects. The Centers for Disease Control and Prevention (CDC) estimates that an average of 1 in 110 children in the United States has an ASD. As part of the same CDC study, the prevalence rate for the New Jersey sites was established at 1 in 49 children.

Governor's Council for Medical Research and Treatment of Autism

The Governor's Council for Medical Research and Treatment of Autism (Council) was created by State appropriation in 1999 and has been issuing research, clinical and educational enhancement grants since 2000. The Council is in the New Jersey State Department of Health (NJ DOH).

As detailed in P.L. 2007, c. 168, the Council is to establish an Autism Center of Excellence in the State. The mission of the New Jersey Autism Center of Excellence (NJ ACE) is to research, apply and advance best practices in the understanding, prevention, evaluation and treatment of autism spectrum disorders, enhancing the lives of individuals across their lifespans. As per P.L. 2007, c.174, monies from \$1 surcharges on fines and penalties from traffic violations are deposited by the State Treasurer into the Autism Medical Research and Treatment Fund to sponsor the Council to fund autism research and treatment in the State of New Jersey.

As part of its enabling legislation, the Executive Director of the Council has the responsibility for establishing a Scientific Advisory Committee (SAC). The SAC includes three biomedical research scientists with demonstrated achievements in biomedical research relating to autism and two medical clinicians whose practice is primarily devoted to the treatment of individuals with autism. The SAC will identify and make recommendations, through the Executive Director, to the Council regarding grants. These recommendations by the SAC are intended as guidance to the deliberations of the Council, which is responsible for decisions on funding of programs and grants.

Council Membership

Elizabeth K. Bell, BS, Autism Organization Representative; Volunteer and Independent Contractor with Autism Speaks.

Matthew Cortland, BA, Public Member; Co-founder and Program Director, BrosUnited (BUD), a mentorship program pairing fraternity brothers with teenage boys with autism.

***Caroline Eggerding, M.D.**, Healthcare Organization Representative; Executive Vice President of Pediatric and Adolescent Services for Bancroft NeuroHealth.

Susan P. Evans, Ed.D., Commissioner of Health and Senior Services Appointee, and the Education Program Specialist for Early Intervention program in the DHSS.

B. Madeleine Goldfarb, MA, Autism Organization Representative; Founder/Director of the Noah's Ark Institute.

Ketan Kansagra, M.D., FAAP, Academic Institution Representative; Division of Neonatal Medicine, Children's Hospital of New Jersey at Newark Beth Israel Medical Center.

Linda S. Meyer, MPA, Ed.D., BCBA-D, Autism Organization Representative; Executive Director of Autism New Jersey.

Barbara J. Morvay, MA, Academic Institution Representative; Member, The Richard Stockton College of New Jersey and author of "My Brother is Different – A Siblings Guide to Coping with Autism".

Grace M. Reilly, RN, MSN, APN-C, Individual with Autism or Family Member Representative; Adult Nurse Practitioner for Riverview Medical Center.

Gary Weitzen, BA, Autism Organization Representative; Executive Director of POAC (Parents of Autistic Children).

Judah Zeigler, BS, the Senate President Appointee, Associate Vice President of Sharp's Retail & Consumer Marketing Group and a father of an autistic son.

* Denotes Council Chair

Council Staff

Martin T. Zanna, M.D., MPH, Acting Executive Director, Governor's Council for Medical Research and Treatment of Autism

Linda N. Bocclair, M.Ed., MBA, Executive Assistant, Governor's Council for Medical Research and Treatment of Autism

Mary Ray, Contract Administrator II, Governor's Council for Medical Research and Treatment of Autism

**Governor's Council for Medical Research and Treatment of Autism
Basic and Clinical Sciences Research Grantees Meeting
National Conference Center at the Holiday Inn
East Windsor, New Jersey
March 23, 2012**

AGENDA

9:30 am Opening Remarks.....Martin T. Zanna M.D., MPH
Acting Executive Director
Governor's Council for Medical Research and Treatment of Autism

9:40 am Welcome..... B. Madeleine Goldfarb, MA
Member, Governor's Council for Medical Research and Treatment of Autism

9:45 am Autism Research: Strengths and Needs in New Jersey.....Dorothy Gaboda, M.S.W., Ph.D.
Rutgers Center for State Health Policy

10:30 am Report of Autism Council Activities.....Martin T. Zanna M.D., MPH
Acting Executive Director
Governor's Council for Medical Research and Treatment of Autism

10:45 am Interdisciplinary Discussion Groups..... Michael Lewis, Ph.D.
Institute for the Study of Child Development, UMDNJ-RWJMS

Group Leaders: *Emanuel DiCicco-Bloom, M.D. (Group 1), Dennis Carmody, Ph.D. (Group 2),
Yvette Janvier, M.D. (Group 3).*

Group 1: What are ways of identifying early onset of ASD? Who identifies and how?
How can basic science research advance our understanding of this topic?

Group 2: What do we know a child with ASD can and cannot do and how do we know it
(i.e. the nature of deficits and strengths)? How can basic science research
advance our understanding of this topic?

Group 3: What do we do to help (i.e. the when and how of interventions)? How can basic
science research advance our understanding of this topic?

12:30 pm Lunch

1:30 pm Group Reports/Discussion

2:45 pm Closing remarks

3:00 pm Adjournment

Opening Remarks

Martin T. Zanna M.D., MPH, Acting Executive Director, Governor's Council for Medical Research and Treatment of Autism

Caroline Eggerding, M.D., Chairperson, Governor's Council for Medical Research and Treatment of Autism

I am pleased to represent the Governor's Council for Medical Research and Treatment of Autism at this forum for the basic and clinical sciences research grantees funded by the Council. Unfortunately, our Council Chair, Dr. Caroline Eggerding, is unable to be present. However, she asked me to read this short note: *Thank you so much for contributing your time and talents to this one day conference on Autism Council Funded Research Grants. I hope that you will be energized by the information you hear today and by the opportunities for collaboration in autism research.*

The Autism Council recently heard the results of a detailed needs assessment that will be used to inform the Council's strategic plan to support research and treatment for autism. Dr. Dorothy Gaboda from the Rutgers Center for State Health Policy will be presenting a summary of the report this morning.

The results of this assessment showed clearly that New Jersey and its citizens with autism will benefit from a strength-based unified research agenda that decreases fragmentation, allows thought leaders in the state to work collaboratively and links New Jersey efforts to national research objectives. This meeting is an important step in achieving that goal.

Thank you for your participation and for your current and future contributions to our understanding of autism.

I am pleased to introduce Madeleine Goldfarb. Ms. Goldfarb is the Founder and Executive Director of The Noah's Ark Institute. She has long been an advocate for children and families with autism and a dedicated and active member of the NJ Governor's Council for Medical Research and Treatment of Autism. Most recently she spearheaded efforts to acknowledge the successes of New Jersey autism researchers, with her efforts culminating in today's meeting. In addition, she serves on many other boards and councils. Particularly noteworthy are:

- The Centers for Disease Control's (CDC) Learn Signs - Act Early State Teams
- The Advisory Board of the Daniel Jordan Fiddle Foundation and
- Her recent appointment to work with FEMA (Federal Emergency Management Agency)

Welcome

B. Madeleine Goldfarb, MA
Member, Governor's Council for Medical Research and Treatment of Autism
Executive Director, Noah's Ark Institute

I am very delighted and pleased to welcome you all.

It is an honor to have the opportunity to be among such a distinguished gathering of scientists, council members, public and staff who have visibly demonstrated their zeal and commitment to the cause of autism treatment and research.

It has been my great pleasure to serve on the Governor's Council for Medical Research and Treatment of Autism, and set in motion this meeting which will lay the framework for future gatherings of the research community. I must thank the Council and Council members for their leadership and vision as we move forward with new and exciting initiatives. Present are fellow members, Ms. Liz Bell, Dr. Susan Evans and Dr. Linda Meyer. Also I wish to convey a very special thanks to the researchers who planned the interdisciplinary discussion sessions for all their work in bringing this meeting from mere idea to fruition. I would be remiss not to acknowledge the unbelievably hard work of the Council's Executive Assistant, Linda Boclair. This meeting would not have been possible without her help and talent.

I hope you will find this day enjoyable and an opportunity to learn more about the work of your colleagues and of the Council. For you to be able to continue your good work, as in most things in life, it takes revenue. For those who are not aware, the funding mechanism, which enables the Council to provide the grant dollars to you, is very interesting. I thought I would share it with you. The mechanism is that of a one dollar surcharge on moving violations in the State of New Jersey. This puts the flow of dollars outside of the traditional State budget and is therefore reliant on the ill-advised habits of drivers on New Jersey roadways, as opposed to more traditional revenue sources.

The revenue for this Council has been remarkably consistent over the years. This may say more about how hard it is to change bad driving habits than anything else, yet, this has afforded the autism community in New Jersey an unprecedented opportunity to shape and encourage the highest caliber of research found anywhere in the country, as evidenced here in this gathering.

As a Council we are keenly aware of the inherent fragility of the monetary situation in a difficult economy and want to present to the Governor, as well as the legislature, a report on the State of the State of Governor's Council funded autism research from the past few years. We are your staunchest advocates. We also want the research community in New Jersey to work

toward greater sharing of ideas, and will continue to encourage this as we move forward. This meeting is an opportunity for you all to engage and discuss, as well as engender your creativity.

We fervently believe in you and your work and want to give you the voice your work deserves with an interest in showing that you have been successful in your endeavors. We shine light on those projects which have been able to maximize their efforts and have gone on to bring revenue beyond the Council's dollars to the State of New Jersey.

You have chosen to work in the field of autism research for various reasons. Perhaps the Council has even had a hand in that decision making process. I represent those of us for whom there was no choice in joining this fight. We are the parents of all the children and adults living everyday with autism. For us there is not respite nor break nor rest... save that of sleep and perhaps even dreams. We live, and obsess and love every aspect of our precious children. We hope against hope and are filled with the sense of urgency that unanswerable questions gives rise. We look to you to make sense of what is so hard to understand.

As we advocate, for our loved ones, we must write our own narrative. We must chart our own course. You, the scientific community are not only our collaborators in fellowship, you are our compass. We look to you to guide our decisions, we entreat you to continue to work tirelessly on our behalf. We are inundated by the deluge of media and modern technology which brings us all the information of an impatient planet without filter or regard for truth. You work to give us that truth. You must go where the scientific method takes you. You must follow the observations of your craft. You are the arbiters of what we know and we put full faith in your capable hands.

We have set you upon a course yet you must lead us to the answers we so desperately seek.

Together we are stepping into the future. We trust that the future will yield all the answers that have been thus far elusive.

We are brimming with confidence regarding the future of autism research and we are here to help tell your story. We wish you a successful meeting. Thank you very much for your presence! In closing, I will leave you with one quote from Margaret Mead..."Never doubt that a small group of caring dedicated people can change the world...In fact, it is the only thing that ever has." I encourage you all to participate actively in the interesting discussions over the course of the day. I wish everyone a successful and fruitful meeting.

Autism Research: Strengths and Needs in New Jersey

Dorothy Gaboda, MSW, Ph.D.
Rutgers Center for State Health Policy

The objectives of this presentation:

- Briefly review national funding priorities – IACC and Autism Speaks
- Overview autism-related funded research in New Jersey from 2005 to 2010
- Share information from follow-up interviews with researchers and other experts
- Suggest some areas of potential opportunity for increasing autism-related research in New Jersey

IACC

The IACC provides direction for ASD-related research activities of US Department of Health and Human Services. Recommended funding approached \$1.2 billion in 2011. The studies of risk factors and treatments and interventions had the highest recommended funding amounts, followed by biology and diagnosis. These four areas represented 79% of the total recommended funding for 2011. Research on autism throughout the life span was added as a new area in 2010 because of the increasing recognition that children with autism are becoming adults living with autism.

Autism Speaks Science Funding

Autism Speaks is the second largest funder of autism-related science and seeks to complement federal funding by focusing on more innovative projects, translation of scientific results into real world outcomes, and dissemination of knowledge to families and the public. Research grants of over \$3 million were awarded nationally in 2009 in each of the three following subject areas: etiology, biology, and treatment and over \$2.5 million for diagnosis. The 3-year strategic plan includes goals to enhance collaboration among researchers from different disciplines to identify interactions between genetic and environmental risk factors and to understand the underlying biological mechanisms of autism.

Autism-related Grants to New Jersey Researchers, 2005-2010

We were able to identify over 80 grants awarded to NJ researchers from 2005-2010, many of them with multiple years of funding. We catalogued over \$103 million in ASD-related funding, although about 70% of that was for tissue repository. NJ researchers received funding from a broad range of sources, both public and private, although the bulk was from the National Institutes for Health.

All of the major academic institutions have researchers receiving autism funding in a variety of areas, immunology at NJIT; genetics, brain function, and neurobiology at Princeton; those areas

plus psychology at Rutgers. UMDNJ was responsible for the bulk of funding, with studies in many areas and at all three medical schools. UMDNJ, Rutgers, and Princeton received funding for training and mentoring of developing researchers.

Aside from funding for tissue repository, the great bulk of the funding has been for genetics and neurobiology, since NJ is home to a number of well-known researchers in these areas. However, there are also prominent researchers in other areas, such as immunology.

Interview respondents mentioned particular strengths of autism research in NJ. Many basic science researchers are well-established and have ongoing national funding. In addition, we heard examples of researchers who made good use of Governor's Council funding to explore new areas and advance their autism research portfolios through additional federal funding. Increasingly, collaboration is important to move autism research ahead nationally, so it was very encouraging that we also heard examples of researchers at different institutions collaborating. Most respondents felt that more collaboration would help to devise more innovative research. Science researchers have had successful collaborations with clinical practitioners also and many said that they would welcome more. Several clinical centers in NJ have participated in research activities such as pharmaceutical studies and collaborations with autism centers in other states, so there is certainly experience to build on in clinical collaboration, depending on the ways which can be found to support it.

Opportunities

Some areas were suggested as opportunities for expanding autism research in NJ.

- Study prevalence, diagnosis, and treatment of ASDs in underrepresented and underserved groups. NJ has great diversity of population within a manageable geography, which is an advantage compared with many other states. We not only have ethnic diversity, but immigrants from a variety of regions and individuals of different economic and educational status. Within the state, there are variations in the ability to access needed care.
- There is a big gap in information about adults with autism, a relatively new priority area for autism research and one that is expected to expand.
- The influence of environmental risk factors on ASDs has received attention in NJ. Both the IACC and Autism Speaks have priority objectives to study interactions between genetic, immune system, and environmental factors.
- Improve early identification of ASDs in very young children. New tools are being developed for use with very young children. Clinical centers in NJ have been working to improve early identification of children, particularly from underrepresented groups, so they are interested in useful tools. That will have the added benefit of increasing the

children who may be able to receive treatment and participate in autism research from an early age.

- Develop a NJ cohort of baby siblings of children with autism, similar to the Early Autism Risk Longitudinal Investigation (EARLI) Study. Recent evidence suggests that the likelihood of families with one autistic child having subsequent children diagnosed with autism is higher than previously thought. Studying these families can have many advantages in finding ways to prevent and treat autism.
- Build on recent findings about the developmental plasticity of teens with ASD by developing a diverse cohort of young adults to devise and pilot interventions focused on meaningful outcomes like educational attainment, independent living, employment, etc.
- Some NJ researchers have done research on medical conditions which are frequently comorbid with autism, such as gastrointestinal problems, sleep problems, and depression, anxiety, Obsessive-Compulsive Disorder (OCD), and other mental health issues. More evidence regarding effective treatment approaches is needed.
- Increase the number of studies using brain imaging in connection with other approaches, e.g., linking brain function and behaviors.

Suggestions from other Autism Centers:

- Effective collaboration between partners is key to success.
- Research is more effective when combined with comprehensive, multidisciplinary care.
- Participating institutions must make a commitment to autism as an area of focus.

Report on Autism Council Activities

Martin T. Zanna M.D., MPH, Acting Executive Director
Governor's Council for Medical Research and Treatment of Autism

Madeline has already explained in more detail the intent of this meeting including: (1) promoting the excellent work conducted by many of you here today with dollars supplied by Council funding by sharing information about your projects and outcomes and (2) initiating active dialogue among yourselves that hopefully will lead to development of new collaborations to further move New Jersey's autism research agenda in a positive direction.

At the end of the day, we hope to increase awareness of New Jersey's wide range of research activities funded by the Council. New Jersey's "portfolio" is diverse in terms of having funded studies ranging from neurobiology, epidemiology, genetics and imaging studies and also the new funding of clinical research.

The continued accomplishments of the researchers in this room are impressive as evidenced by the reports you've sent us, your publications and your ability to attract additional funding by foundations and governmental agencies. We applaud you for your accomplishments.

In reviewing Council's minutes, I see the idea for this conference was engendered as early as June 2011 by Council's discussion and with Madeline's help as well as Linda Bocclair, Council Chair Dr. Caroline Eggerding and researchers Drs. Michael Lewis, Emanuel DiCicco-Bloom, Dennis Carmody and Yvette Janvier. While unknown at the time, another state (South Carolina) was also developing a similar scientific meeting held in the late fall of 2011. This was spearheaded by their federally funded Translational Research Institute. We do hope New Jersey's meeting will further catalyze similar energy to stimulate collaborative efforts among clinicians and basic science researchers in New Jersey to facilitate interdisciplinary, multi-institutional collaborations. The intent is to connect basic and clinical researchers to further this cause. Having this meeting is quite innovative. New Jersey, without such an Institute as mentioned above, is quite ambitious in sponsoring this event, made possible with the help of the Council dollars.

As an aside, today's agenda is also timely in that with Congress's recent re-authorization of the Combating Autism Reauthorization Act last September there was inclusion of language to stimulate new collaborative research projects among clinical and basic science researchers.

In the remaining time, let me highlight a few autism related initiatives that have been undertaken by the Council:

Scientific Advisory Committee (SAC)

The SAC convened its first meeting by telephone on February 15, 2012. The SAC is mandated as part of the New Jersey Autism Biomedical Research Act (P.L. 2007 c.168). It's primary role is to identify and make recommendations, through the Executive Director, to the Council regarding grants. These recommendations by the SAC are intended as guidance to the deliberations of the Council, which is responsible for decisions on funding of programs and grants.

SAC Members include:

Ted Abel, Ph.D.

Professor of Biology
Director, Biological Basis of Behavior Program
Center for Autism Research, Translational Research Center
University of Pennsylvania, Philadelphia, PA

Joseph D. Buxbaum, Ph.D.

Professor of Psychiatry, Neuroscience, Genetics and Genomic Sciences
Director of the Seaver Autism Center for Research and Treatment
Mount Sinai School of Medicine, New York, NY

Susan L. Hyman, M.D.

Professor of Pediatrics
Division Chief, Neurodevelopmental & Behavioral Pediatrics
Golisano Children's Hospital
University of Rochester Medical Center, Rochester, NY

Stewart H. Mostofsky, M.D.

Associate Professor of Neurology and Psychiatry
Johns Hopkins University School of Medicine
Director, Laboratory for Neurocognitive and Imaging Research
Medical Director, Center for Autism and Related Disorders
Kennedy Krieger Institute, Baltimore, MD

Catherine E. Rice, Ph.D.

Developmental Disabilities Branch on Detail with Prevention Research Branch
National Center on Birth Defects and Developmental Disabilities
Centers for Disease Control and Prevention (CDC), Atlanta, GA

Visits to the Basic Science Researcher's Sites:

Visits to two of the basic science grantees were a great opportunity for me, representing the Council, to see first-hand how the labs function, speak candidly with the researchers about the progress of their research and learn how significant Council's funding is in building a research team. The 2010-2012 Basic Science and Clinical Research grant program funds research primarily in the areas of genetics, fMRI/brain development and neurobiology. The Council's

2010-2011 Biannual Report includes details of the grant program and is posted at www.nj.gov/health/autism.

NJ Autism Center of Excellence (NJ ACE)*

The Council is committed to advancing the current knowledge pool through clinical research that can lead to improvements in interventions that address the physical and behavioral health needs of children, adolescents and adults with ASD.

In June 2012 the Council, through the New Jersey Department of Health, will fund the NJ Autism Center of Excellence (NJ ACE), consisting of up to three Program Sites and a Coordinating Center. The Program Sites will conduct clinical research projects that address the national priorities described in the Interagency Autism Coordinating Committee (IACC) Strategic Plan. If applicable, the applicants will also reference Healthy People 2020 autism-related objectives addressed by the research project. The Coordinating Center will provide common management and support functions to unify the NJ ACE Program Sites by serving as the voice of the NJ ACE and promoting the sharing of lessons learned and best practices in the conduct of clinical research.

To advance the goal of widespread data sharing among ASD researchers, the National Institutes of Health (NIH) National Database for Autism Research (NDAR) will function as a data repository for all NJ ACE clinical research projects. The data and results gained by using the Council's funds will allow investigators from New Jersey to develop stronger proposals for submission to the NIH and biomedical research foundations. Council awards for this funding cycle are intended to promote clinical research, not to provide long-term support. Funding will be awarded in June 2012 with annual renewals through May 2017, based on availability of funds and grantee performance.

*Update: In June 2012 the NJ ACE Coordinating Center grant was awarded to Montclair State University. In addition, by June 2013 three Program Sites and six Pilot Project grants were awarded to hospitals, universities and medical schools to fund clinical research projects, totally over \$12M.

The Clinical Enhancement Centers

The Clinical Enhancement Center (CEC) grant initiative enabled six clinical autism centers across New Jersey to enhance staffing of clinical personnel, improve early identification of ASD and increase the number of multi-disciplinary evaluations provided to children suspected of being on the autism spectrum. The third year of a three-year cycle ended on June 30, 2011 with three of the six Centers continuing with no cost extensions through June 30, 2012.

Funding totaled \$8.5M for the three year period (July 1, 2008 – June 30, 2011). Council funding resulted in increased accessibility to diagnostic and treatment services through the addition of new clinical programs and specialized medical personnel and expanded hours of operation. In the third year of funding 5,414 patients received care at the six Clinical Enhancement Centers with a total of 15,370 patient visits. Diagnostic autism evaluations were conducted for 2,570 patients, resulting in 1,131 diagnoses of ASD.

The six Centers also conducted extensive outreach and education programs to primary care physicians to improve screening and early identification efforts across the State. In addition, the Centers provided outreach to pediatric residency programs, nurse practitioners, daycare providers and preschool teachers. The Centers also developed education and information resources such as a regional autism telephone answer line and a website listing autism services and provider information. Summary reports are posted at www.nj.gov/health/autism.

Research Funding in FY 13

The Council intends to provide, based on the availability of funds, up to \$6M for autism research in FY 13. An ad hoc committee of the Council has been charged with recommending the categories of research grants to be funded in FY13.

Further Comments

Because it is difficult for many of you to come to our Council meetings because of your busy schedules, we endeavor to keep our stakeholders, such as yourselves, informed via our Council's website (www.nj.gov/health/autism). You will find information on:

- Membership
- Annual Reports
- Minutes of Council Meetings
- Funding Opportunities
- Resources
- Meeting announcements/calendar
- Contact information

Thank you for your time and please feel free to speak with me individually in-between your sessions.

Interdisciplinary Discussion Introduction, Summation and Recommendations

Michael Lewis, Ph.D., Institute for the Study of Child Development, UMDNJ-RWJMS
Emanuel DiCicco-Bloom, M.D., Dept. Neuroscience & Cell Biology/Pediatrics, UMDNJ-RWJMS
Dennis Carmody, Ph.D., Institute for the Study of Child Development, UMDNJ-RWJMS
Yvette Janvier, MD, Children's Specialized Hospital

Introduction by Michael Lewis, Ph.D.

The goals of the discussion groups are (1) to achieve a better understanding of what researchers know about Autism Spectrum Disorders (ASD) and what can be learned from each other and (2) to encourage clinical and basic science investigators to explore the kinds of information each has that is relevant to the other and how to take the next step toward working together.

Each group has been assigned questions to guide the discussions.

Group 1 Questions: What are ways of identifying early onset of ASD? Who identifies the children and how? How can basic science research advance our understanding of this topic?

Reporting: Emanuel DiCicco-Bloom, M.D., Dept. Neuroscience & Cell Biology/Pediatrics, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

Members: Jill Harris, Ph.D., Xue Ming, M.D., Ph.D., Robert G. Nagele, Ph.D., T. Peter Stein, Ph.D., Ankur Patel, M.D. and Renping Zhou, Ph.D.

This group consisted mostly of basic scientists and a clinician, Dr. Jill Harris from Children's Specialized Hospital (CSH). Dr. Harris was the one member who routinely sees children with autism. She was asked to address the following questions: "What are you doing at CSH? How are you identifying children with ASD? What new tools could more basic scientists provide you with to inform the process of identifying children in the clinic with ASD?" These questions served to provide a context for establishing a productive clinician-basic scientist interactive dialogue.

Dr. Harris discussed the various programs at CSH, including the outreach and education programs for community members, educators and healthcare providers as the first step in creating an awareness of ASD and the resources available if ASD is suspected. She described the services at CSH including the diagnostic screening clinic and follow-up procedures. The goal is to identify children with ASD as early as possible to provide options for treatment.

She then explained the tools clinicians use at CSH for the early identification of autism and what challenges clinicians face with the tools used to assess children who are 12 months or older. The group discussed the need for improved tools and how improved and/or new tools might be

engendered by collaborating with basic science in the preliminary identification of the tools in a translational sense.

One of CSH's goals is to develop new tools that might be more sensitive to the realities and/or the environmental constraints of the children being evaluated/screened. CSH is interested in tools that are subject to less bias (i.e. based on cultural experiences and less emphasis on literacy) such as those tools that involve visual learning as opposed to tools that are subject to linguistic bias toward particular sub-populations.

Among members of the group were a geneticist, an expert working with mouse models, two people studying biomarkers (one related to the immune system and one related to metabolic indicators) and a clinician. The questions addressed were: (a) "How can we speak to each other?" (b) "Can we do collaborative research emphasizing sub-populations?" The discussion then turned to how the clinicians and basic scientists can work together with the fundamental strategy to facilitate a common understanding, that outcomes/pathways identified by the basic scientists might serve a dual purpose; (1) eventually such pathways can be used to intervene for therapy while (2) others may just be a biological marker for risk. For example, as is known from the clinical literature, abnormalities in eye movement are one of the indications for early diagnosis of possible problems with social interactions. More specifically, basic science has information that may help understand how to subcategorize individuals whether it's an early biomarker (vision used in mice), a gene in the mouse model or a metabolic pathway studied in people and mice.

A brisk conversation engendered related questions: (c) "What else can we introduce to this population to bring the basic science oriented researchers and the clinicians together to address the question of early identification of ASD?" (d) "Is collaboration possible (and consistent with IRB policies, NIH research criteria, and state regulations) whereby the basic scientists collect biological samples for research from the clinic patients?" (e) "Would the families agree to providing samples?" Collection of swabs for DNA or urine samples would be one option and seems to be non-threatening as compared to finger sticks or venipuncture for blood samples that are more invasive. A question was asked about the use of urine samples. Dr. Ming, a national expert on the subject, stated that there are a number of tests using urine specimens. Urine concentrates so there is value in testing urine for metabolic markers including oxidative stress as a biomarker. An advantage is these samples are less invasive, easy to obtain and logistically better samples for these studies.

Further, if the basic science researchers could obtain samples for DNA profiling, for example, they could look for risk genes or stratify individuals within the patient population that is well characterized. This is a strategy for identifying a gene or several genes associated with a particular pathway while other genes are not associated. They then look for correlation

between genes across particular characteristics, including social, language, intellectual development or even drug responses. In similar fashion the same kind of analysis can be done on metabolic products such as urine or finger sticks for antibody levels to look for antibody biomarkers, although finger sticks may be too invasive, and no consistent antibody markers are currently known. The idea emerging from the discussion of being able to obtain biological samples was felt to be very useful. Some of the participants exchanged cards and phone numbers so as to start collaborations.

As an added note, Dr. Harris emphasized the importance of communication that considers the patients' and their families' perspectives particularly with respect to the question of "what is in it for them". It was suggested that the investigators talk prospectively to the community about the value of the work and the importance of patient participation in the studies.

With a stable clinical infrastructure in the state, the basic science researchers could, in follow-up to this conference, develop stronger ties with the clinicians and the investigators, particularly those who are doing early identification. There are many clinicians in the state with many patients but it is unlikely that there are sufficient resources to allow individuals collecting human data to have a data set that can actually be optimized for research. The clinical demands are great and the priority is the delivery of patient care, not organizing the patient populations. Resources would be needed to allow clinical and basic scientists to collaborate on pilot and exploratory/collaborative grants.

One idea worthy of consideration is the creation of a biological materials resource center. The state is collecting blood samples at birth with the heel stick for the purpose of performing 50 different tests. The approach, if the state supported the idea, would be proactive in that the samples would be collected in a way that could be archived for biological testing, not limited to genetic testing. Although there are privacy issues, a reasonable place to start would be with a discussion of possibilities. California has been performing heel sticks for 30 years and has a model that can be referenced for discussion.

It is also true that the level of skill needed (people who have created large family samples and understand the parameters, IRB and archiving) is essential. It was suggested that consideration be given to bringing clinical research expertise to New Jersey by funding a Chair in autism, someone with successful NIH clinical autism research, including translational.

In general, New Jersey researchers may not be ready to collaborate nationally (particularly across disciplines). The group discussed pilot studies and translational research that Council might consider supporting. While the state is viewed as limited in their readiness for national projects, translational research within New Jersey would be an option and an alternative to

build capacity. It was suggested that supplemental funding could be provided to the current clinical centers to work with basic science researchers.

It was also suggested that funding fellowships would increase capacity ensuring a stable future for autism research in New Jersey with investigators initiating and following through with studies. Dr. Harris commented on the difficulty in trying to back fill the clinical time spent and commented that fellowship programs are very important avenue to fill this gap. Fellows have the energy, commitment and motivation needed for sustainability. Training someone who continues to work on the research ensures longevity to projects.

“What are the barriers to doing what has been discussed and how can they be addressed?” One institutional barrier can be IRBs that are very demanding and time consuming. Other barriers will probably require deans, vice presidents and others at the institutions and members of the Governor’s Council and/or state to collaborate in addressing barriers, whether real or perceived.

Group 2: What do we know a child with ASD can and cannot do and how do we know it (i.e. the nature of deficits and strengths)? How can basic science research advance our understanding of this topic?

Reporting: Dennis Carmody, Ph.D., Institute for the Study of Child Development, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

Members: Gabriella D’Arcangelo, Ph.D., Abby Hare, M.S. representing Linda M. Brzustowicz, M.D., Jill Harris, Ph.D., James H. Millonig, Ph.D., and Walter Zahorodny, Ph.D.

One clinician in the group commented that given that ASD is a heterogeneous disorder there is no classification based on what any particular child can or cannot do – it depends. Then the question was asked “What would the basic scientist need to know about children to develop some type of a characterization?” There was a consensus from the basic scientists that they need some kind of a homogenous sample in order to know what to look for as a specific gene to knock out in a mouse model. And they wanted better classifications of particular phenotypes so they could model.

One candidate was the tubular sclerosis research by Dr. Gabriella D’Arcangelo. Many of the children with this disorder are about 50% comorbid with ASD. This may then define a particular subsample. There is one institution in New York and one in Philadelphia collecting samples. Dr. D’Arcangelo commented that to conduct autism research she would add a team to test for ASD and begin looking at other aspects within this particular clinical sample given that they

have a high degree of epilepsy and also simple genotyping in the mouse model. She can have a mouse model of this particular disorder and then treat for epilepsy and also follow surgical removal of parts of the brain that are dysfunctional. This may be a particular model but this disorder is not highly prevalent in autism although autism with tubular sclerosis is highly prevalent.

The basic scientists wanted to know if tests such as ADOS and ADI can be done every six months to see how well a child progresses. In clinical practice every time an ADOS is repeated on a child there is another child on a four month waiting list that then increases to five or six months. To obtain good developmental change over time requires added expense for repeated measures for a subset of patients; the clinician cannot repeat tests for children while increasing the wait time for other children who are in need of initial testing.

A way of identifying and recording change over time is needed, if it is not the ADOS. Also, the genetics researchers need a better way of coding the data; when data is recorded there frequently are differences in how the clinicians present the case. It is very difficult to find consistency over time or to look at consistency of findings over laboratories.

It was suggested that one way of identifying children, at least in the beginning, is to look at IQ; three groups (under 70, 70-85 and over 85) to determine whether they have stereotypies or not, what type of socialization they have (highly deficient or not) and if they have epilepsy. With these characterizations basic science researchers working with mouse models would be able to develop equivalents. While the group did not find a way of doing that with communication, there is a way with socialization problems.

One researcher described performing EEGs on children as they were screened using ADOS as a way to characterize the children who have particular biological signatures of brain which can be modeled by biological signature of EEG in mouse models. It is also possible to use MRI to determine the areas of overgrowth and undergrowth in children in developmental stages and look at those equivalent developmental stages in the mouse model to determine what could be done to create (or not) the overgrowth in those particular areas.

It was suggested that for the basic scientist it may be easier to look at the adult with ASD and look at the adult mouse model. That way there is a stable end point. However; that is not what clinicians see in the clinic and the priority is in finding those preschoolers who need interventions before they get to that point. So often times using those types of imaging studies to talk about the autistic brain is not a clear characterization of what clinicians are faced with in the screening situation. So the question then is how to get brain signatures on the developing child that could parallel brain signatures in the developing mouse and know what types of

interventions can be used in the mouse to change and alter those signatures and then see if that parallel can be brought to the clinical setting.

There was a discussion about allowing multiple principal investigators on grant applications rather than just one principal investigator. That would allow collaboration not only across disciplines but across institutions within the state such that both get credit and then can perhaps combine two particular grants into one with have a wider base.

Group 3: What do we do to help (i.e. the when and how of interventions)? How can basic science research advance our understanding of this topic?

Reporting: Yvette Janvier, M.D., Children's Specialized Hospital

Members: Bonnie L. Firestein, Ph.D., Gleb Shumyatsky, Ph.D., Samuel S. Wang, Ph.D., Harvey R. Weiss, Ph.D. and Michael Lewis, Ph.D.

The group had quite a range of expertise and discussed some of the same processes that the other groups discussed including blood flow in the cerebellum and proteins that affect dendrite functions. The group decided to answer the assigned question in the context of their best fantasy world, starting with a discussion of the critical period of bringing change and improvement in a child with autism. If that period could be expanded, a blood test or any kind of a test would be needed to identify a child at birth who would be at risk. If the period of sensitivity could be expanded pharmacologically so it didn't matter if the child was identified at 2 or 3 or 4 years of age then it would improve that time to respond to interventions.

There seem to be so many different pathways to autism and there are many areas of research. It is very important to know the cause of autism and the effect and how to go back and treat it. Both the upstream and downstream central nervous system effects need to be identified. Which comes first, the chicken or the egg? Pharmacologically, interventions that were effective (they may be very different at critical time periods) are very important. It could be something like in the fragile X model in Dr. Mark Bear's research (Massachusetts Institute of Technology). Regulation and deregulation of certain genes are reported to have a curative effect and this is very important and in the future will be a hot area of research. And then there is the correlation of genes to behavior and connectivity to early identification – it is very important to identify that linkage.

Dr. Samuel Wang commented that it became apparent during the discussion that there were very different kinds of analyses from functions of synaptic proteins all the way up to blood flow abnormalities in animal models and issues seen in children both normal and also those with autism. Given all these very different models of analysis, something that basic research can

contribute is a framework for starting to link the ideas together. Which brain regions might be causative and which might be more downstream consequences? Critical periods and sensitivity are fundamentally developmental in nature and they overlap quite a lot. The group heard about development in the report from Group 2. Following these mechanisms upstream is going to be very helpful in understanding both the human disorder and finding a point of commonality with the animal models with the potential to join all of these topics together into a framework.

There was a lot of enthusiasm about pharmacological interventions. Once something is known about the brain regions that are defective it becomes something more like what is seen in Parkinson's disease with deep brain stimulation/DBS (inserting a probe in a targeted area of the brain to address the defect). There is a distinct link in that many of the disparate findings and what has been discussed can be put together by this last point about understanding brain regions and their connections.

Comments and summary-Dr. Michael Lewis

Dr. Lewis commented on the need for a more homogenous group to study. What is needed is a classification system to provide a way to identify a homogeneous group; from genes, from hormones, in terms of brain function. The idea of rather than taking the broad perspective of many children potentially with ASD, focusing more specifically on a particular group may be advantageous. Co-morbidity with some other disorder may be a way of identifying that group. It is important not so much for early identification but in that process of early identification, rather than a clinical activity, a research activity to try to produce a homogeneous group. That requires the cooperation and collaboration across many different clinical centers to get a large enough sample. Homogeneity is a critical issue because in all of the discussions the idea of an inadequate classification system in terms of this disorder is a deficient that interferes with any of our attempts to bring basic science to bear in a most productive way.

Another idea in terms of biobanks of collecting biological materials is potentially a kind of commitment that requires an interdisciplinary effort within the clinical setting with the cooperation of families and children. The idea is to create within the state a longitudinal study of bringing children and families into the sample and then have a standardized set of measures to be collected and to follow. To bring children 10 years of age or maybe 15 years - 20, 25, 30 children a year would provide a rather large sample in each clinical center across the state. The individual child or family cooperating would have a standard set of measures and materials to be collected where it would be bagged until such time that the samples build up. Working with the state has significant advantages because it speaks to some effort in New Jersey and it speaks to a collaborative nature.

What is also interesting is the idea of fellowships so in every clinical center not only is there a data collection activity but an education/training activity as well. The issue about longitudinal studies is that in a lifetime a researcher can only do one or two longitudinal studies. One problem is that once the study is complete there is no money to analyze the data. There was funding to collect the data, the investigators are now aged and with no more funding and no one to continue the research, all of the resources are lost. Combining the research with an educational component as well as commitment is the best approach.

That kind of effort has many of the advantages that this meeting has tried to accomplish and that is to get basic sciences and clinicians together so clinicians working in the clinic know that they have a group of basic scientists and clinicians collecting these kinds of materials.

Summary of recommendations:

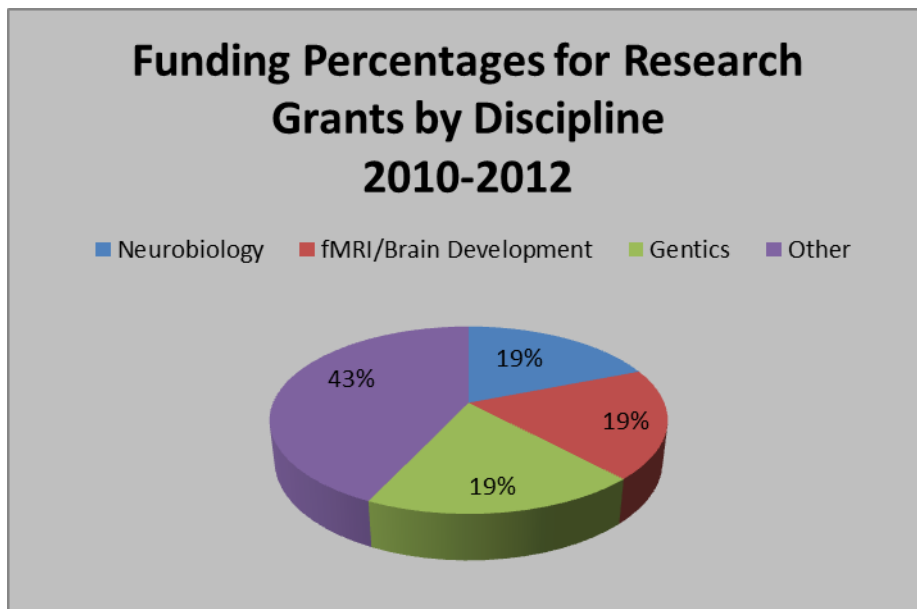
1. Funding fellowships would increase capacity ensuring a stable future for autism research in New Jersey with investigators initiating and following through with studies.
2. Provide resources to allow clinical and basic scientists to collaborate on pilot and exploratory/collaborative grants, including translational research.
3. An idea worthy of consideration is the creation of a biological materials resource center that would archive blood samples collected at birth to be used for biological testing and research.
4. It was suggested that consideration be given to bringing clinical research expertise to New Jersey by funding a Chair in autism, someone with successful NIH clinical autism research and in creating human populations for genetic studies.
5. Allow multiple principal investigators on grant applications rather than just one principal investigator. That would allow collaboration not only across disciplines but across institutions within the state such that both get credit and then can perhaps combine two particular grants into one with a wider base.
6. Given all the very different models of analysis, something that basic research can contribute is a framework for starting to link these ideas together.

Funding Initiatives

Basic Science and Clinical Research Grants

In July 2010 the Council awarded 11 two-year Basic Science and Clinical Research grants for a total of \$5 million. The grant program funded research primarily in the areas of genetics, fMRI/brain development, and neurobiology. Accomplishments of the grantees are impressive with significant progress in accomplishing the goals of the projects as evidenced by publications in professional peer reviewed journals. Grantees have also received additional funding through federal agencies and foundations, another indication of the quality of their research. Grantees' progress reports, including accomplishments, are posted at www.nj.gov/health/autism under "Current Initiatives".

The following chart illustrates the percentage that each of these disciplines was funded during this grant cycle:



2010 – 2012 Basic and Clinical Science Grantees
***no cost extensions through 2013**

Principal Investigator	Title	Institution	Type of Study	Funding Level
Bharat Biswal	Altered brain connectivity in Autism Spectrum Disorder patients	*UMDNJ-NJMS	Basic Science	\$469,550
Gabriella D’Arcangelo	Reelin abnormalities in synaptogenesis and ASD	*Rutgers University	Basic Science	\$381,700
Emmanuel DiCicco-Bloom	En2 Regulates Forebrain Monoamines and Behavior	*UMDNJ-RWJMS	Basic Science	\$469,550
Bonnie Firestein	Regulation of synaptogenesis by Cypin and Neuroligin-1	*Rutgers University	Basic Science	\$469,550
Yvette Janvier	Autism outreach /screening in under-served areas	Children’s Specialized Hospital	Clinical Research	\$469,550
G. Miller “Mill” Jonakait	Mediators of maternal inflammation	*NJIT	Basic Science	\$392,162
Michael Lewis	Self representation and brain Development in Autism	*UMDNJ-RWJMS	Clinical Research	\$469,550

James Millonig	Genetic and epigenetic Engrailed-2 ASD risk factors	*UMDNJ-RWJMS	Basic Science	\$469,550
Maggie Shiffrar	Perceptual-motor anticipation in individuals with Autism Spectrum Disorder	*Rutgers University	Clinical Research	\$469,550
Gleb Shumyatsky	Amygdala-enriched genes in behaviors Related to ASD	*Rutgers University	Basic Science	\$469,550
Harvey Weiss	Autism and control of cerebral metabolism	*UMDNJ-RWJMS	Basic Science	\$469,550

The Clinical Enhancement Centers

The Clinical Enhancement Center (CEC) grant initiative enabled six clinical autism centers across New Jersey to enhance staffing of clinical personnel, improve early identification of ASD and increase the number of multi-disciplinary evaluations provided to children suspected of being on the autism spectrum. The third year of a three-year cycle ended on June 30, 2011 with three of the six Centers continuing with no cost extensions through June 30, 2012.

Funding totaled \$8.5M for the three year period (July 1, 2008 – June 30, 2011). Council funding resulted in increased accessibility to diagnostic and treatment services through the addition of new clinical programs and specialized medical personnel and expanded hours of operation. In the third year of funding 5,414 patients received care at the six Clinical Enhancement Centers with a total of 15,370 patient visits. Diagnostic autism evaluations were conducted for 2,570 patients, resulting in 1,131 diagnoses of ASD.

The six Centers also conducted extensive outreach and education programs to primary care physicians to improve screening and early identification efforts across the State. In addition, the Centers provided outreach to pediatric residency programs, nurse practitioners, daycare providers and preschool teachers. The Centers also developed education and information

resources such as a regional autism telephone answer line and a website listing autism services and provider information. Clinical Enhancement Center summary reports are posted on the Council’s website at www.nj.gov/health/autism under “Current Initiatives”.

2008-2011 Clinical Enhancement Centers
***no cost extensions through 2012**

Principal Investigator	Institution	Total Funding (3 Years)
Mark Mintz, M.D.	The Center for Neurological and Neurodevelopmental Health Gibbsboro, New Jersey	\$ 1,500,000
Yvette M. Janvier, M.D.	Children’s Specialized Hospital Toms River, New Jersey	\$ 1,500,000
Randy Huron, M.D.	*Hackensack University Medical Center - Institute for Child Development, Hackensack, New Jersey	\$ 1,500,000
Audrey Mars, M.D.	*Hunterdon Medical Center – Child Development Center, Flemington, New Jersey	\$ 1,050,000
Denise Aloisio, M.D.	Jersey Shore University Medical Center, Neptune, New Jersey	\$ 1,500,000
Tyrone Bentley, M.D.	*University of Medicine and Dentistry, New Jersey Medical School Newark, New Jersey	\$ 1,500,000

NJ Autism Center of Excellence (NJ ACE)*

The Council is committed to advancing the current knowledge pool through clinical research that can lead to improvements in interventions that address the physical and behavioral health needs of children, adolescents and adults with ASD.

In June 2012 the Council, through the NJDHSS, will fund the NJ Autism Center of Excellence (NJ ACE), consisting of up to three Program Sites and a Coordinating Center. The Program Sites will conduct clinical research projects that address the national priorities described in the Interagency Autism Coordinating Committee (IACC) Strategic Plan. If applicable, the applicants will also reference Healthy People 2020 autism related objectives addressed by the research project. To advance the goal of widespread data sharing among ASD researchers, the NIH National Database for Autism Research (NDAR) will function as a data repository for all NJ ACE clinical research projects. The data and results gained by using the Council's funds will allow investigators from New Jersey to develop stronger proposals for submission to the National Institutes of Health (NIH) and biomedical research foundations. Council awards for this funding cycle are intended to promote clinical research, not to provide long-term support. Funding will be awarded in June 2012 with annual renewals through May 2017, based on availability of funds and grantee performance.

The Coordinating Center will provide common management and support functions to unify the NJ ACE Program Sites by serving as the voice of the NJ ACE and promoting the sharing of lessons learned and best practices in the conduct of clinical research. Funding will be awarded in June 2012 with annual renewals through May 2017, based on availability of funds and grantee performance.

*Update: In June 2012 the NJ ACE Coordinating Center grant was awarded to Montclair State University. In addition, by June 2013 three Program Sites and six Pilot Project grants were awarded to hospitals, universities and medical schools to fund clinical research projects, totally over \$12M.

Research Funding in FY 13

Council intends to provide, based on the availability of funds, \$2M for autism research in FY 13. An ad hoc committee of the Council has been charged with recommending the categories of research grants to be funded, with a final report due in September 2012 and Council vote in December 2012.

In Memoriam to Steven Zalcman, Ph.D.

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Brain, Behavior, and Immunity

journalhomepage:www.elsevier.com/locate/ybrbi

In Memoriam

In memoriam Steven S. Zalcman (1956–2011)



Steve Zalcman passed away suddenly on Sunday, December 25, 2011 while on vacation with family in Florida. Family, colleagues and friends will all attest that there was hardly a day on which Steve did not carry a large briefcase filled with data to analyze, manuscripts to write, grants to develop, and papers and grants to review. His last vacation, itself a rare event, was no exception. Steve was a full time scholar whose mastery of psychology, neuroscience, and neuroimmunology were masterfully integrated in an all-too-brief, stellar career in psychoneuroimmunology (PNI). He was passionately devoted to PNI, its staunch defender from international meetings to the halls of NIH, a passion that extended to and engaged many friends and colleagues. Steve was fascinated by the behavioral effects of cytokines and by the neurochemical mechanisms of those effects. That fascination is reflected in a wave of compelling and seminal publications, beginning in the laboratory of Hymie Anisman in 1991 and yet to come to rest. At least nine formal publications remain in process with students and colleagues. Steve's most recent funded research, primarily with mouse models, include studies of IL-2 effects on stereotypic behavior and the role of dopaminergic receptors; effects of virus-induced immune activation in pregnancy on autism-associated neurobehavioral disturbances in offspring; the role of sexual dimorphism and developmental stage in IL-2 effects on behavior

and the HPA-axis; and the role of serotonin and cytokines in the neural circuitry and the neurochemical and neurophysiological mechanisms of aggression.

Steve Zalcman was tenacious and extraordinarily careful in his approach to experimental problems. He approached his writing with the same tenacity and care, his papers reflecting impeccable scientific standards. Steve was much admired and respected by his colleagues, students, research assistants and, really, anyone with whom he made contact. He was a sought after and welcomed collaborator and mentor within his own institution, the UMDNJ New Jersey Medical School, and throughout the world. It would be difficult to find a research colleague, co-faculty member or trainee who is not reminded of the 5–10 min brief scientific question that ended an hour or two later, punctuated by Steve's hilarious scientific anecdotes and resulting in a new direction for research. We were not surprised to learn recently that, as an undergraduate at McGill, Steve wrote comedy, much of it satirical and philosophical, and performed in a comedy troupe. After winning a Canadian Broadcasting radio competition, he was offered a professional contract, which, to the benefit of science, he declined. Other scholarly roads that he traveled before those of psychology, neuroscience, and neuroimmunology, and which clearly contributed to his incisive and expansive science, included those of Genetics and Philosophy at McGill, and, in high school, the paths of Talmudic logic. As he completed college, Steve was accepted into a doctoral program in Philosophy. He also briefly considered going to law school. Fortunately for us, science won out over all. Steve, as a Canadian, naturally loved hockey and, growing up in Montreal, the Canadiens. He played on street hockey teams and in more formal leagues until a young adult. As an undergraduate, he coached a soccer team of underprivileged children from the league cellar to a championship. He wrote novels and short stories for fun, as well as to hone his writing skills. And Steve loved music. He loved classic rock and jazz and acquired an encyclopedic knowledge of those genres. He was a solid guitarist and hosted a popular night-time program on Radio McGill.

Steve was born in Montreal to very caring parents, survivors of the Holocaust who raised him and his sister Dorothy to love people and knowledge. After a rigorous Jewish Day School education and his undergraduate studies at McGill in Genetics and Philosophy, he elected to do another Bachelor's degree in Psychology, at the University of Ottawa. There he met the late Howard S. Rosenblatt, Professor of Psychology at the University of Hartford, a pivotal teacher and mentor, who encouraged Steve to complete a Master's in Neuroscience in Hartford. Steve then returned to Ottawa to pursue a Ph.D. in Psychology/Behavioral Neuroscience with Hymie Anisman at Carleton University, with a focus on PNI. His graduate work resulted in some of the first reports on central changes in catecholamines during immune challenge and during stress-induced suppression of innate immunity. In 1990, Steve joined the laboratory of the late Arnold Greenberg at the University of Manitoba as a postdoctoral fellow. At Manitoba, he met Dwight Nance, a mentor and colleague with whom he developed a strong and continuing professional relationship. Dwight recalls Steve's arrival in the middle of the Manitoba winter, enthusiastic,

with a head full of ideas. The lab was publishing on conditioning of responses to cytokines and other immune stimuli, on sympathetic innervation of immune organs, and on the brain effects of stress, pharmacologic and neuroanatomical manipulation, and immune activation. With his already established interest in the behavioral and neurochemical effects of cytokines, Steve undertook the first systematic examination of the differential effects of cytokines on central monoamines, and discovered the behavior activating effects of interleukins. This work served as the foundation for the research program that emerged through his career. In a typical (among Steve's favorite) elegant series of experiments, he demonstrated that IL-2 could enhance the plaque-forming-cell (PFC) response, that the effect was mediated by b-adrenergic receptors and that it was mediated via the sympathetic splenic nerve.

Following his post-doc, Steve returned briefly to Ottawa and then accepted a faculty position in the Department of Psychology at Concordia University in Montreal. In 1998, we had the great fortune of recruiting him to the Department of Psychiatry at the UMDNJ-New Jersey Medical School in Newark, where he established an exceptional basic science program in PNI. Steve was also active in the school's Interdisciplinary PhD program and in the Rutgers-UMDNJ Integrative Neuroscience Program, mentoring numerous pre-doctoral students and post-doctoral fellows. He became a key member of our largely clinical Department of Psychiatry, admired and sought after by clinical Residents as a teacher and research mentor, and appointed Director of Research in the department in 2009.

Steve's expertise, his respect for science and for colleagues, and his exceptional common sense made him a highly regarded member of NIH Study Sections and of the Editorial Board of BBI. His colleagues recall him as an outstanding champion of PNI research on study sections that often had only limited understanding of the field. Important funded research in PNI might never have happened if not for Steve's erudite and articulate advocacy. And he stayed with the task, even at the cost of having less time for his own work. Keith Kelley, who served on an NIH study section with Steve and then asked him to serve on the editorial board of BBI in 2006, noted his insightful, inquisitive mind and his knack for recognizing good science, as well as his warm, welcoming smile and infectious love for biomedical research. Recently, Steve co-edited "The Neuroimmunological Basis of Behavior and Mental Disorders" with Allan Siegel, a fitting expression of the scope of his interests. His trail-blazing collaboration with Siegel on cytokines and aggression has transformed that field, most recently elucidating TNFalpha effects on aggressive behavior. At the time of his death, Steve was especially excited about findings concerning effects of soluble IL-2 and IL-6 receptors on stereotypic behaviors and on the role of anti-streptococcus IgM and dopamine in mediating stereotypic movements. The first report, by Steve and his colleagues, on the specific role of IgM in precipitating unique behavioral disturbances is presented elsewhere in this issue of BBI (Zhang et al., 2012).

Steve Zalzman devoted himself fully and unconditionally to the people he loved, the activities he valued, and the ideas he cherished. He inspired those around him, opening many minds to new ways of thinking and perceiving. He was individualistic and meticulous, with an unquenchable curiosity and an elegant mind. He challenged conventions with intellectual and personal integrity. Steve was taken from us as he was approaching the peak of his career. Tragically, his creativity and productivity is now lost to the scientific, and especially the PNI, community. We have lost a great colleague and friend. Those of us who had the privilege of knowing him can only be grateful for that opportunity. Steve is survived by his sister and her family who live in Montreal. We will miss him greatly.

Allan Siegel

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Appendix

- List of Participants
- Participants' Evaluation of the Meeting
- Research Reports

**NJ Governor's Council for Medical Research and Treatment of Autism
Scientific Meeting of Basic and Clinical Science Research Grantees
March 23, 2012**

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**Governor's Council for Medical Research and Treatment of Autism
Basic and Clinical Sciences Research Grantees Meeting
March 23, 2012**

Participants' Comments

"This was an excellent meeting. I would suggest that you send out emails about international/national meetings for autism research." Bonnie Firestein, Ph.D., Rutgers University.

"Thank you for inviting me. Enjoyed very well structured and attended meeting!"

"Very productive regarding understanding of the areas of research in NJ and how they can interact. Thank you."

"Excellent meeting. Thank you."

"I thought the meeting was very constructive and interesting. Thanks for making it possible. I hope it can be done on a regular basis." Walter Zahorodny, Ph.D. UMDNJ-New Jersey Medical School

"The meeting was a great start to developing a dialogue between NJ autism researchers and clinicians. I hope this can be done regularly."

"The meeting was an excellent start. More of this is necessary. Some really good ideas. Thank you for putting it together."

"Great to interact with other researchers in NJ."

"The best part of the meeting was talking to others doing similar things." Harvey Weiss, Ph.D., UMDNJ-Robert Wood Johnson Medical School

"Well organized conference by the Council. Multi-institutional effort. Recommendations: Support for establishing a clinical and biological repository. Support for clinicians contributing to research."

"Very informative; opens some doors for me (planning and collaboration); very happy to see this energy pushing this forward; looking forward to bring basic scientists and clinical folks closer together; would like to see a priority for biomarkers for early detection and risk determination." Robert Nagele, Ph.D., UMDNJ-School of Osteopathic Medicine

“Include more clinical grantees in discussion groups. While the discussions were informative, inclusion of more clinicians will help to make discussions more realistic as opposed to idealistic means of data collection”.

“For next workshop have some presentations in the AM to inform discussion groups, presenting salient clinical and basic research questions.”

“Developing collaborations between clinicians and basic scientist is difficult. Casual meeting between basic scientists and clinicians (who usually do not attend the same meetings) is a rather haphazard process. The commission has the potential to fill a role here by identifying common interests, clinical and analytical and using this information to stimulate the formation of large integrated groups.” Peter Stein, Ph.D., UMDNJ-School of Osteopathic Medicine

“It seems very important to link levels of analysis – developmental, animal vs. human, basic vs. clinical. Basic research can contribute a framework for understanding the fundamental systems - neuroscience of autism. Two priorities: 1. Understanding sensitive periods and 2. Identifying brain regions that are upstream causes and downstream consequences. I was asked to be on the Council two years ago, willing to serve.” Sam Wang, Ph.D. Princeton University

“If you allow multiple PIs on grants, maybe allowing collaboration with PIs outside of NJ? This would allow better interactions, still utilizing NJ institutions.” Gleb Shumyatsky Ph.D., Rutgers University

“Recommendations: \$1.5 million invested for autism chair, generates \$150,000 for salary and basic scientists on Council from NJ; out of state.” Manny Diccico-Bloom MD, UMDNJ-Robert Wood Johnson Medical School

*Recorded from written comments at the meeting and emails following the meeting.
L. Boclair*

Research Reports

Gabriella D’Arcangelo, Ph.D.
Rutgers, the State University of New Jersey

Emanuel DiCicco-Bloom, MD
UMDNJ-Robert Wood Johnson Medical School

Bonnie L. Firestein, Ph.D.
Rutgers, the State University of New Jersey

Yvette Janvier, MD
Jill Harris Ph.D.
Children’s Specialized Hospital

Michael Lewis, Ph.D.
Dennis Carmody, Ph.D.
UMDNJ-Robert Wood Johnson Medical School

James H. Millonig, Ph.D.
UMDNJ-Robert Wood Johnson Medical School

Xue Ming, M.D., Ph.D.
UMDNJ-New Jersey Medical School

Robert G. Nagele, PhD
UMDNJ – Stratford

Nicholas M. Ponzio, Ph.D.
UMDNJ-New Jersey Medical School

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Principal Investigator: Gabriella D’Arcangelo, Ph.D.

Title: Reelin abnormalities in synaptogenesis and ASD

Project Period: 7/1/2010 – 6/30/2012

Abstract: 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 revealed 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 this proposal we will study how these proteins affect the development of spines in the brain of mutant mice. We will also examine whether specific autism-associated synaptic proteins are abnormal in mutant mice deficient in Reelin activity to determine whether these mice are viable models for the disease.

Highlights of the research (past and present): In 1995 I identified Reelin, a gene initially implicated in neuronal migration during early brain development and associated with autism. I then contributed to the discovery of the signaling transduction pathway that mediates this function, and helped to identify Dab1 as an adapter protein that is essential for Reelin activity. In following years my laboratory demonstrated that Reelin is also important for other aspects of postnatal brain development, such as neuronal maturation and synapse formation. With the support of this grant we recently demonstrated that Reelin affects the molecular composition of the excitatory synapse in the developing as well as the adult brain. We also showed that Dab1 deficiency affects the molecular composition of excitatory synapses in the developing

brain in a similar manner. Together, these findings suggest that Reelin and Dab1 play an important role in synapse formation, a process that may be disrupted in autism.

Publications and Presentations:

Ventruiti A, Domogauer J, Kazdoba TM, and D’Arcangelo G. The Role of Reelin in Forebrain Synapse Development. San Diego, CA: Society for Neuroscience, 2010. Program No. 134.11. (Abstract of poster presentation)

Ventruiti A, Kazdoba TM, Niu S and D’Arcangelo G (2011) Reelin deficiency causes specific defects in the molecular composition of the synapses in the adult brain. *Neuroscience*, 189, 32-42.

Kazdoba TM, Sunnen CN, Crowell BC, Lee GH, Anderson AE, and D’Arcangelo G. Development and characterization of NEX-Pten, a novel forebrain excitatory neurons-specific knockout mouse. *Dev Neurosci* (in press).

D’Arcangelo G. Regulation of dendritogenesis and spine formation by Reelin. Invited lecture in the symposium “ApoE, Alzheimer’s and lipoprotein biology”, 40th Keystone Symposia on Molecular and Cellular Biology, Keystone, CO, February-March 2012.

Research activities:

We are investigating the function of the Reelin signaling pathway in synapse formation in the developing and adult mouse brain. Reelin is a ligand that can trigger a molecular signaling cascade that regulates many aspects of brain development, including neuronal migration, dendrite maturation and synapse formation. Using confocal imaging and biochemical techniques we are analyzing synapse formation in the developing brain of mutant mice in which Reelin signaling is disrupted. These include heterozygous *reeler* mice, expressing reduced Reelin levels at different ages, and conditional knock out mice in which the essential transducer Dab1 is deleted specifically in the adult brain (cDab1 knock out mice). We focused on the analysis of the hippocampus, a brain region important for learning and memory. Our recent data indicate minimal abnormalities in the density of synapses in adult *reeler* as well as in cDab1 knock out mice, suggesting that this signaling pathway is not required for the maintenance of synaptic structures in the adult brain. However, we found significant abnormalities in the molecular composition of the synapses in *reeler* mice, particularly in the expression of postsynaptic proteins such as the scaffolding PSD-95 protein and subunits of the NMDA receptor, which is important for synaptic function and plasticity. We are currently analyzing synapse formation and the expression of synaptic proteins in Dab1 knock out mice, and examining the expression of synaptic proteins that have been previously implicated in autism in both our animal models.

Preliminary data indicate that autism-related proteins such as Neuregulins and Neurexins are not altered in *reeler* mice. However, we discovered that the synaptic expression PTEN, a phosphatase encoded by an autism-associated gene, is reduced in mutant mice, and that this proteins interacts with the NMDA receptor and PSD-95 at the synapse. These findings prompted us to examine the levels of NMDA receptor subunits and Dab1 in *PTEN* mutant mice. For this purpose we characterized novel conditional knock out *Pten* mice in which the gene is specifically deleted in excitatory neurons of the forebrain. We discovered that the expression of NMDA receptor subunits and Dab1 is deregulated, suggesting that a Reelin- and Pten-dependent signaling events cross-talk and affect excitatory synapse formation.

Significance of the research to the field of autism:

Cognitive and behavioral disorders such as autism are thought to result from defects in brain cell connectivity and synaptic function that occur during development. Current research from several laboratories further suggests that alterations in the excitatory/inhibitory balance may be a potential mechanism of disease. Thus, it is essential to identify signals that promote and regulate the formation of excitatory and inhibitory synapses. These signals may then be targeted for modulation to ameliorate autistic symptoms in patients.

How the findings advance the field of autism:

Our studies demonstrate that the Reelin signaling pathway affects the molecular composition of excitatory synapses in the postnatal brain. Of particular interest are the reduction we found in the levels of NMDA receptor subunits at the synapse, which could profoundly affect synaptic function and plasticity. The abnormalities that we identified in our animal models may also be present in some autistic patients, implicating these molecular mechanisms in the etiology of the disease.

The future directions for this line of research:

We will complete the molecular analysis of synapses in Reelin and Dab1 mutant mice, and examine potential changes in synaptic proteins that have been directly implicated in autism by human genetic studies. If we identify specific autism-related proteins that are affected by disruptions of the Reelin-Dab1 signaling pathway we will pursue the identification of the molecular mechanisms leading to this defect. Key components of the Reelin signaling pathway will then be targeted in an attempt to restore the normal pattern of expression of autism-related synaptic proteins, and thus restore normal brain function.

Other Support:

Pending Research Support (recommended for funding)

Exploration-Hypothesis Development Award #TS110033, D'Arcangelo (PI), 9/30/12-9/29/14

Department of Defense – CDMRP - TSCR P

Exploring the interaction between TSC2, PTEN and the NMDA receptor in animal models of tuberous sclerosis

The goal of this project is to determine whether TSC2 and PTEN mutations affect the expression of NMDA receptor subunits

Emanuel DiCicco-Bloom, MD

Professor

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Principal Investigator: Emanuel DiCicco-Bloom, MD

Title: En2 Regulates Forebrain Monoamines and Behavior

Project Period: 7/1/2010 – 6/30/2012

Abstract: 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 norepinephrine. 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.

Research activities: Aim 1: studies completed. Aim 2: TH fibers, a marker of locus coeruleus (LC) innervation, in hippocampus are reduced by 75%, and other LC markers, NE transporter and galanin, are also reduced, indicating altered forebrain monoamines in En2 KO reflects abnormal axon growth. Since LC neuron number is unchanged, fiber pathfinding may be disturbed. Abnormal hippocampal NE is associated with abnormal neurogenesis, with increased cell death (apoptosis), loss of dentate gyrus neurons and increased (compensatory?) proliferation; both apoptosis and proliferation now serve as biomarkers. Changes in brain volumes by MRI are being correlated with stereological neuron counting. Aim 3: En2 KO mice exhibit reduced juvenile and adult social behaviors, and deficient cognition (water maze; cued and contextual fear), but no changes in repetitive behaviors or ultrasonic vocalizations.

Significance of the research to the field of autism: These basic studies demonstrate that NE innervation of the forebrain depends on normal En2 function in the hindbrain. Brain size, weight, neuron numbers, ongoing neurogenesis and autism-related behaviors seem to depend on normal monoamine development. The EN2 disease associated AC allele may contribute to the disorder by altering monoamine development and function. In addition, these studies suggest that we need to develop methods to detect human NE levels in ASD, so that we can consider using related drugs for therapy.

How the findings advance the field of autism: Since En2 has been reproducibly found to be genetically associated with ASD, these studies suggest that more attention be paid to evaluating the function of NE neurotransmitter systems in patients. Indeed, related drugs, especially propranolol (Inderal), a NE blocker, has known clinical efficacy. Together this evidence may spur further research. The studies also now raise a new hypothesis in ASD pathogenesis, that disease related genes may alter the brain by causing abnormal regulation of neurogenesis, including cell death (apoptosis) as well as cell proliferation in the hippocampus. Normal hippocampal neurogenesis is required for normal learning and memory, which are abnormal in many with ASD as well as the En2 KO mouse model.

The future directions for this line of research: Using our insights into reduced NE levels in the forebrain, our research is moving in two directions. First, we plan to use NE related drugs to correct NE deficits and determine whether dysregulated neurogenesis, including apoptosis and proliferation, are reversed. In parallel, we are using the same drugs to determine whether such treatment can rescue the abnormal behaviors including social interactions and cued and contextual fear.

Resulting publications:

1. Genestine, M., Lin, L., Yan, Y., Prem, S., Millonig, J.H., and E. DiCicco-Bloom, E. (2011) Absence of Engrailed 2 (*En2*), the Autism Spectrum Disorder (ASD) Associated Gene, Alters Monoamine Transmitter Systems, Forebrain Structure and Developmental Neurogenesis and Apoptosis. 10th Annual International Meeting for Autism Research (IMFAR), San Diego, CA, May 12-14, 2011.
2. Brielmaier, J., Silverman, J.L., Lin L., Matteson, P.G., Kamdar, S., Millonig, J.H., DiCicco-Bloom, E. and Crawley, J.N. (2011) Selective Social Deficits in Engrailed-2 mutant mice. 13th Annual Meeting of the International Behavioral and Neural Genetics Society, Rome, Italy, May 11-14, 2011.
3. M. Genestine, L. Lin, Y. Yan, S. Prem, P. Sonsalla, J. H. Millonig and E. DiCicco-Bloom (2011) Absence of *Engrailed 2 (En2)*, the Autism Spectrum Disorder (ASD) associated gene, alters locus coeruleus fiber elaboration and hippocampal neurogenesis and apoptosis. Society for Neuroscience, November, 2011, Washington DC.
4. Brielmaier, J., Silverman, J.L., Lin, L., Matteson, P.G., Kamdar, S., Millonig, J.H. DiCicco-Bloom, E., and Crawley, J.N. (2011) Autism-related behavioral phenotypes in *Engrailed-2* mutant mice. Society for Neuroscience, November, 2011, Washington DC.

SUBMITTED MANUSCRIPT: Lin, L., Yan, Y., Sonsalla, P.K., Matteson, P.G., Silverman, J., May, V., Crawley, J.N., Millonig, J.H. and DiCicco-Bloom, E. Absence of Autism Associated Gene *Engrailed-2 (En2)* Produces Deficits in Forebrain Monoamines and Regional Structures and Depression-like Behaviors.

Other Support:

Pending Grants:

1. DOD, Congressionally Directed Medical Research Program, Autism Research Program; Pilot Grant # AR110227; 2 years.
Title: Define the Ability of Norepinephrinergetic Therapy to Rescue an Autism-Related Animal Model
2. Simons Foundation: Pilot Grant Program, Letter of Intent submitted; 2 years.
Title: Neurobiological mechanisms and therapeutic strategies in *Engrailed2* mutants
3. Autism Speaks, Translational Postdoctoral Fellowship Program, Letter of Intent submitted; 2 years.
Title: Forebrain development and monoamine therapy in *Engrailed 2* mutant mice

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Principal Investigator: Bonnie L. Firestein, Ph.D.

Title: Regulation of Synaptogenesis by Cypin and Neuroligin-1

Project Period: 7/1/2010 – 6/30/2012

Abstract: We are studying how cypin and NLGN-1 regulate PSD-95 localization and excitatory synapse formation and whether there is crosstalk between the two proteins. To summarize, overexpression of cypin causes a decrease in the synaptic localization of PSD-95. Knock down of cypin results in increased synaptic targeting of PSD-95, and this increase cannot be rescued by a cypin mutant that cannot bind PSD-95. Our new data suggest that overexpression of cypin also affects total PSD-95 levels and that it is necessary for activity-dependent turnover of synaptic PSD-95. Furthermore, our data suggest that cypin increases ubiquitination of PSD-95 and acts via a proteasome-dependent pathway to regulate PSD-95 levels. We have also found that overexpression of NLGN-1 results in upregulation of cypin. Thus, we have made progress in our understanding of how cypin regulates PSD-95 levels and its relationship to NLGN-1 signaling.

Significance of the research to the field of autism:

In order for the brain to function, neurons must be able to communicate properly through connections called synapses. There must also be a balance of excitatory and inhibitory information passing through the synapses. Synapse formation, a process called synaptogenesis, must therefore be tightly regulated during development because aberrations in this process can lead to cognitive dysfunction. In patients with autism and autism spectrum disorders (ASD), this delicate balance is disrupted. Therefore, understanding how synaptogenesis is regulated will yield insight into at least some of the mechanisms underlying autism and ASD. This work studies three main proteins that are involved in autism and ASD. First, PSD-95 is an integral component of the excitatory synapse. It clusters neurotransmitter receptors and associated signaling molecules. When PSD-95 levels are altered, excitatory receptors do not function properly, and hence, there is an aberrant ratio of excitatory to inhibitory input. In fact, variations in the human PSD-95/DLG4 gene are associated with phenotypes relevant to autism and ASD, and PSD-95/DLG4 knockout mice show abnormalities relevant to autism and ASD. Second, mutations in genes encoding NLGNs and their cognate receptors, neuroligins, have been linked to ASD. NLGN-1 increases PSD-95 clusters at synaptic sites. Third, cypin is an enzyme that

breaks down compounds called purines, a process that is often altered in patients with autism and ASD. Cypin decreases PSD-95 at synapses. The question remains as to how to treat autistic patients to restore excitatory/inhibitory synaptic balance. Our research will identify the mechanisms by which PSD-95 protein levels are regulated, either by NLGNs or by cypin.

Resulting publications and presentations:

Kara Mann, Vaishali Kulkarni, Hyuck Kim, Yue Zhuo, and Bonnie L. Firestein. Regulation of Synaptogenesis by Cypin. Autism New Jersey, 29th Annual Conference, Atlantic City, NJ, October 13-14, 2011. (Poster Presentation)

Munjin Kwon, Yue Zhuo, Kristina Hernandez, and Bonnie L. Firestein. Cypin regulates PSD-95 levels via a proteasome-dependent pathway, in preparation.

Other Support:

None at this time although we have submitted grant applications based on this work to various agencies.

Research activities:

We are studying how cypin and NLGN-1 regulate PSD-95 localization and excitatory synapse formation and whether there is crosstalk between the two proteins. To summarize, overexpression of cypin causes a decrease in the synaptic localization of PSD-95. Knock down of cypin results in increased synaptic targeting of PSD-95, and this increase cannot be rescued by a cypin mutant that cannot bind PSD-95. Our new data suggest that overexpression of cypin also affects total PSD-95 levels and that it is necessary for activity-dependent turnover of synaptic PSD-95. Furthermore, our data suggest that cypin increases ubiquitination of PSD-95 and acts via a proteasome-dependent pathway to regulate PSD-95 levels. We have also found that overexpression of NLGN-1 results in upregulation of cypin. Thus, we have made progress in our understanding of how cypin regulates PSD-95 levels and its relationship to NLGN-1 signaling.

How the findings advance the field of autism:

Our data suggest that the construction of pharmacological agents that disrupt the binding of cypin to PSD-95 may be an option for restoring balance to the abnormal levels of PSD-95 caused by mutations in PSD-95 itself or in NLGNs in autism or autism spectrum disorders.

The future directions for this line of research:

We will complete the mechanistic analysis of cypin- and NLGN-1-promoted changes in PSD-95 levels and localization. A manuscript describing our results on these data is currently in preparation, and will be submitted within the next 6-9 months.

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Principal Investigator: Yvette Janvier, MD

Title: Autism outreach/screening in underserved areas

Project Period: 7/1/2010 – 6/30/2012

Abstract: Although no racial or ethnic differences have been found in the epidemiology or phenotype of autistic disorder, research has revealed both racial and income disparities in the early detection and treatment of autism. The purpose of this study is to increase the early identification of ASD in traditionally underserved young, low income, minority children and facilitate their entry into early intervention services. Autism education workshops will be held for healthcare providers in five target communities in New Jersey with large low income and minority populations. As part of these workshops, healthcare providers will be trained to administer the M-CHAT (a Level 1 developmental screening) to children in their practices. In partnership with community leaders, culturally-sensitive outreach materials will be created to cover normative child development, behavioral signs of possible developmental delay and resources regarding access to child health services. These materials will be used to provide workshops in easily accessible locations throughout these communities in order to impart information regarding autism 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 in order to determine the autism risk status of children referred by community healthcare or education providers, parents/guardians, or early intervention staff. Risk status will be based on Level 1 screening when necessary, as well as on the STAT (a Level 2 developmental screening), additional developmental screening tools, clinical observation, and history. Those children in whom either generalized developmental delays or symptoms of ASD are found will be referred to the appropriate Early Intervention Program if younger than 36 months or to the appropriate local child study team for referral to a Preschool Disabled Program if between 36 and 60 months. 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 this study to result in much greater accessibility to diagnostic services and early entry into appropriate early intervention services 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

Resulting publications:

Janvier, Y., Hampton, P., Zuniga, M. & Cable, G. "Feasibility of autism screening in underserved populations". Paper accepted for presentation at the International Meeting for Autism Research (IMFAR), May 2012, Toronto, Canada.

Harris, J., Walpin, L. & Blann, L. "Parent-teacher agreement on autism screeners in an underserved preschool population". Poster accepted for presentation at the International Meeting for Autism Research (IMFAR), May 2012, Toronto, Canada.

Janvier, Y., Harris, J., & Cable, G. Autism screening in underserved areas, poster presented at the International Meeting for Autism Research (IMFAR), May 2011, San Diego, California

Janvier, Y. Autism screening and outreach in underserved areas. Poster presented at Autism New Jersey annual conference, October 2011, Atlantic City, NJ.

Other Support:

Grant personnel salaries are funded in part by Children's Specialized Hospital and in part by this grant.

Research activities:

- 1) *Education of caregivers and other community members.* Ten parent outreach/education workshops were held. Twenty-five training workshops for community-based health care or early childhood staff have been conducted. Staff participated in four community health fairs to raise awareness regarding autism and developmental delays.
- 2) *Community Autism Screening.* Screenings have been conducted at Head Start and other daycare centers in six target cities (an additional city was added from the original grant proposal) using parent and teacher responses on the Modified Checklist of Autism in Toddlers

(MCHAT) and/or Social Communication Questionnaire (SCQ). Parents/teachers of all children in the class were asked to complete the screening, thus this was a general population sample rather than a population sample with known developmental concerns. A total of 1086 children were screened. Analysis has been completed for 877 of the children screened. The most recent 206 screened children are in the process of having follow up interviews and scheduling of research evaluations. Of those 877 with completed scoring, seventeen percent (N=151) screened positive after a follow-up interview and were offered research evaluations to determine diagnosis. Fifty research evaluations were subsequently conducted (33% refused or were lost to follow-up). Twenty-six (52%) of the children who received research evaluations were diagnosed with an autism spectrum disorder. Thus, at least 2.96% (26 out of 877) of the children screened in a community setting were found to have an ASD. Comparisons of parent and teacher screenings were conducted to determine agreement and were then compared to final diagnosis for those children who had a research evaluation.

3) *Autism screening clinics*- Clinics were established at federally-funded health clinics in Bridgeton, Elizabeth, Plainfield, Trenton and Newark. These children are referred based on a developmental concern. Seventy-two were screened through 3/7/2012 in 2011 with 32 (44%) found to have an ASD.

4) *Culturally sensitive materials*- Based on staff observations that many parents did not understand all the items on the MCHAT, we developed a pictorial checklist (“Developmental-Check-In”) and the “Watching my child grow” coloring book. Both of these illustrate early developmental milestones and may be useful as supplements to existing screening tools to increase accuracy of parent report for those parents with literacy, comprehension, cultural or language-based issues. These tools can also be used during community outreach/education sessions to aid understanding.

Significance of the research to the field of autism:

This research has helped to determine modifications that may be needed to reduce income, racial and cultural disparities in accessing early diagnosis of autism and to determine utility of traditional screening tools and outreach materials with this population. Preliminary results suggest there may be a higher incidence of autism (approximately 3%) in this population than the one-percent reported in state or national statistics. Parenthetically, we note that many parents appear to have difficulty linking to community services recommended as a result of the screening or research evaluation.

How the findings advance the field of autism:

Preliminary experiences suggest that 1) literacy and cultural factors may affect utility of commonly used parent-report based screening instruments, 2) multiple contacts (both formal and informal) are needed to establish trust in underserved communities in order to conduct meaningful outreach and autism screening, 3) actual prevalence of ASD in underserved populations may be higher than previous reports in the general state and national population, 4) performing autism screening in preschools/daycare settings is a feasible way to improve access, and 5) parents in this underserved population poorly identify cases at risk for autism, whereas preschool teachers are good at identifying cases at risk for ASD but not as accurate identifying those children not at risk. Thus, including both parent and teacher screening reports increases the accuracy of finding cases of ASD in a preschool underserved population.

The future directions for this line of research:

We plan to hold two working summit meetings for thought leaders in the African-American and in the Latino/Hispanic communities to further ascertain perception of, experiences with and needs related to autism in those communities.

Future research needs include:

1. Further identification of variables which may result in disparities in identification and service delivery for low income, culturally/ethnically/racially diverse, and non-English speaking communities.
2. Identification of models of screening, outreach and service delivery that may overcome identified barriers.
3. Development of screening tools with improved reliability and validity for these populations.
4. Determine effect of supplementary “culture-fair” tools on parent understanding and accuracy of autism screening.
5. Determine feasibility of tele-medicine for autism screening/diagnosis in underserved areas.
6. Compare effect of trained parent-mentor versus professional social worker versus typical community care on linkages to services, parent satisfaction, and child functioning for newly diagnosed families in underserved areas.

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Principal Investigator: Michael Lewis, Ph.D.

Titles and project periods: “Brain Maturation and Self-Representation in Young Children with Autism Spectrum Disorder” (2007-2009) and “Self-Representation and Brain Development in Autism” (2010-2012).

Scientific Abstract

The aim of this project is to study the relations between white matter development in brain and the social, emotional, and cognitive development in children with autism spectrum disorder (ASD). ASD includes a group of developmental disabilities caused by an abnormality in the brain and is characterized by impairment in the ability to interact with others. It is caused, in part, by failures in the development of the child’s self representation and therefore his ability to interact with others. The failure to develop self representation is important to study in that interventions provided early in development may reduce the severity of the impaired social relations. Many children with ASD do not show self-recognition in a mirror, a well-established measure of self representation, long after most children have achieved this developmental milestone. Those children who do not exhibit self recognition have been found to be more likely to lack communicative speech and to be rated lower in overall social functioning. We have published our evidence that the emergence of self representation is related to brain maturation in typically developing children. Pilot data from children with ASD, presented below, shows the relation between brain maturation in specific brain regions and social development. We plan to expand on those findings using state of the art imaging procedures and methods of data analysis. Specifically, we plan to study the development of brain structure in autism using MRI. Children ages 2 to 4 years will undergo MRI while sleeping naturally. Within one month of the MRI, the children will be assessed for self representation abilities and for the severity of their autism. The MRI images will be assessed for regional measures of gray matter and white matter. The imaging measures will be related to the severity of autism as assessed by the

ADOS, as well as to the levels of self representation in each child. In addition, we plan to compare the MRI measures of gray and white matter to the normative database developed by the NIH in their study of 'The MRI Study of Normal Brain Development.'

Publications and Presentations:

Lewis, M., & Carmody, D. P. (2008). Self-representation and brain development. *Developmental Psychology*, 44(5), 1329-1334. doi: 10.1037/a0012681

Carmody, D. P., & Lewis, M. (2010). Regional white matter development in children with autism spectrum disorders. *Developmental Psychobiology*, 52(8), 755-763. doi: 10.1002/dev.20471

Carmody, D. P., & Lewis, M. (October 13-14, 2011). Self Referential Behavior: Basic Research Findings and Clinical Implications. Poster presented at the Governor's Council for Medical Research and Treatment of Autism, Basic Science and Clinical Research Grants, Autism New Jersey 29th Annual Conference, Atlantic City Conference Center.

Carmody, D. P., & Lewis, M. (2011 early view). Self representation in children with and without autism spectrum disorders. *Child Psychiatry and Human Development*. doi:10.1007/s10578-011-0261-2; scheduled for April 2012 release

Kim, N. H., Carmody, D. P., & Lewis, M. (2012). *Self Representation and Frontal Brain Structure in Children with ASD*. To be presented May 17, 2012 at the International Meeting for Autism Research (IMFAR).

Other Support:

We submitted a letter of intent in January 2011 to the Simons Foundation Autism Research Initiative (SFARI).

Research activities:

We have investigated the associations between brain development and social development in children with Autism Spectrum Disorder (ASD). To achieve these goals we conducted behavioral studies of self representation, coded the behaviors for social development and analyzed neuroimaging data of brain development using voxel-based morphometry (VBM) in the same children. We have found that children with ASD were deficient or delayed in self-referential behavior. We have obtained in these children the volumes of gray matter and white matter in frontal brain cortex at the level of Brodmann regions. We tested the hypothesis that increased white matter in frontal brain regions would be associated with deficits in self representation in children with ASD. Strong associations were found between self representation and white matter volume in both left and right whole brain in that greater volumes were associated with

lower self representation scores. Regional level analyses using Brodmann areas (BA) showed that the associations were located in left BA 10 and 11, as well as both left and right BA4 and BA6.

Significance of the research to the field of autism: We have shown the relations of altered brain development with the deficits in social development as well as deficits in self representation in children with ASD. Deficits in self representation result in dysfunctional emotional regulation and emotional expressive behavior including embarrassment, empathy and shame/guilt.

How the findings advance the field of autism: These findings contribute to the growing evidence that brain development is associated with the social deficits in ASD. It also contributes to our understanding of emotional deficits in children with ASD.

The future directions for this line of research: We plan to continue this work by measuring brain development using voxel-based-morphometry (VBM). We hypothesize that the measures of brain development will be associated with the deficits in social development shown by children with ASD. We also believe that social support through interventions will lead to changes in brain structure. In addition knowledge about deficits in brain structure and function will allow us to study the effects of intervention and how intervention affects changes in brain and behavior. In the proposed research we will be able to answer the following questions:

1. Does treatment improve brain development?
2. Are certain brains more likely to be changed by interventions?
3. Does the early brain structure interact with intervention to produce greater outcomes?

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Principal Investigator: James H. Millonig, Ph.D.

Title: Genetic and epigenetic ENGRAILED 2 ASD risk factors (2005-2008) and

Project Period: 7/1/2010 – 6/30/2012

Lay Abstract: Risk to Autism Spectrum Disorder is likely a combination of genetic susceptibility and nongenetic 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.

Highlights of the research (past and present): This project focused on testing other candidate genes in the ENGRAILED 2 pathway for association with ASD. Our research identified the homeobox transcription factor, *ENGRAILED2* (*EN2*), as ASD susceptibility gene. Previous data demonstrated that an intronic *EN2* haplotype (*rs1861972*---*rs1861973* A---C) is over---transmitted to individuals with ASD (518 families, 2336 individuals, P=.00000035). Since then 5 other groups have demonstrated 4 association for *rs1861972* or *rs1861973*. These studies suggest that a DNA variant in *EN2* increases ASD risk. We would expect this risk variant to affect expression or activity of *EN2*. Subsequent genetic studies identified the ASD A---C haplotype as the best candidate to test for function. In vitro analysis has demonstrated that the A---C haplotype functions as a transcriptional activator due to the specific binding of two transcription factors, *CUX1* and *NFIB*. To investigate the function of the A---C haplotype throughout development, we generated transgenic mice. The A---C haplotype results in increased levels and expanded expression domains throughout development and in multiple brain structures relevant to ASD etiology. Finally *EN2* levels are also increased in affected post---mortem samples. Together these data support *EN2* as a susceptibility gene and are consistent with increased levels being correlated with ASD risk.

Publications and Presentations:

Gharani N, Benayed R, Mancuso V, Brzustowicz LM and Millonig JH. (2004) Association of the homeodomain transcription factor, *ENGRAILED 2*, with Autism Spectrum Disorder. *Mol. Psychiatry* 9: 474-484

Benayed R, Gharani N, Rossman I, Mancuso V, Lazar G, Kamdar S, Bruse SE, Tischfield S, Smith BJ, Zimmerman R, DiCicco-Bloom E, Brzustowicz LM, Millonig JH. (2005) Support for the homeobox transcription factor, *ENGRAILED 2*, as an Autism Spectrum Disorder (ASD) susceptibility locus. *Am J of Hum Genetics* 77: 851-868.

Cheh M, Millonig JH, Roselli, LM, Ming X, Kamdar S, Wagner GC. (2006) *En2* Mutant Mice Display Neurobehavioral and Neurochemical Alterations Relevant to Autism Spectrum Disorder. *Brain Res.* 1116: 166-76.

Benayed R, Choi J, Matteson PG, Gharani N, Kamdar S, Brzustowicz LM, Millonig JH. (2009) Autism Associated Haplotype Affects the Regulation of the Homeobox Gene, *ENGRAILED 2*. *Biol Psychiatry* 60: 911-17

Rossman, IT, Lulu L, Malanga K, DiGiovine M, Pasoreck E, Millonig JH, DiCicco-Bloom E.

Altering expression of the autism-associated gene, Engrailed 2 (En2), disrupts cerebellar precursor proliferation, differentiation and responses to insulin-like growth factor (IGFI). (submitted)

Bartlett CW, Gharani N, Millonig JH and Brzustowicz, LM. (2005) Three Autistic Candidate Genes: Synthesis of Human Genetic Analysis with other Disciplines. *Int J Dev Neurobiol* 23:221-234.

Choi J, Kamdar S, Rahman T, Matteson PG, Millonig JH. (2011). *ENGRAILED 2 (EN2)* genetic and functional analysis. Autism Spectrum Disorders - From Genes to Environment, ISBN 978-953-307-558-7, edited by Tim Williams. pp1-22

Choi J, Ababon M, Matteson PG, Millonig JH. (2011) Cut-like homeobox1 and Nuclear factor I/B mediate *ENGRAILED2* Autism Spectrum Disorder-associated haplotype function *Human Molecular Genetics* Epub 12/11

Other Support:

NIMH R21MH083509 Title: A mouse knock-in model for EN2 autism susceptibility

Millonig (PI) (R21/33 MH083509) 4/1/08-3/31/11

National Institutes of Mental Health - Funding supports the generation of knock-in mouse lines for the *EN2* ASD risk allele

Millonig (PI) (R01 MH076624) 10/01/05-9/30/10

National Institutes of Mental Health - Funding supports the identification of other ASD susceptibility loci.

Millonig (PI) 7/1/09-6/30/10

Department of Defense - Funding supports the experiments to investigate the potential epigenetic regulation of *EN2*

Millonig (PI) 7/07-6/09

NARSAD Young Investigator Award – Funding supports additional association analysis for *ENGRAILED 2*

Millonig (PI) 7/06-6/09

Autism Speaks – Funding supports pilot experiments to investigate whether stem cells

can be used to reverse the autistic-like neuroanatomical phenotypes in *Engrailed 2* knockout mice.

Millonig (PI) 1/07-12/08

Autism Speaks - Functional examination of candidate ENGRAILED 2 Autism Spectrum Disorder disease alleles by mouse transgenesis

Millonig (PI) 7/05-12/07 National Alliance for Autism Research – This research funds our further genetic and functional characterization of the *ENGRAILED 2* gene to identify polymorphisms consistent with them being an ASD risk allele(s).

Research activities:

Our previous research demonstrated that *ENGRAILED 2* (*EN2*) is an ASD susceptibility gene. *EN2* is expressed throughout brain development and regulates numerous developmental processes implicated in ASD including connectivity, excitatory/inhibitory circuit balance as well as the development of serotonin, norepinephrine and dopamine neurons. Our genetic studies indicate that the common alleles (underlined) of two intronic SNPS (*rs1861972* A/G; *rs1861973* C/T) are consistently and significantly associated with ASD ($P=.00000035$). Six other groups have also reported association of *EN2* with ASD. These data support *EN2* as a susceptibility gene. To provide additional evidence that *EN2* is a susceptibility gene, we investigated the potential function of the ASD-associated haplotype (A-C *rs1861972-rs1861973*). Using a cell culture system we demonstrated that the ASD-associated A-C haplotype increases gene expression. Both associated alleles are sufficient and necessary for this activator function. In addition the AC haplotype specifically binds a protein complex. These proteins were partially purified and two transcription factors, Nfib and Cux1, were identified. Knock-down, over-expression, antibody supershift, and ChIP demonstrated that CUX1 and NFIB bind the A-C haplotype at the same time and both proteins are required to mediate A-C haplotype function. These studies provide biochemical evidence that the ASD-associated haplotype functions as a transcriptional activator (see Choi et al., 2011 for more details). To investigate whether *EN2* levels are increased in individuals with autism, post-mortem analysis was performed. 90 cerebellar post-mortem samples were obtained and *EN2* levels were measured by QRTPCR. A significant increase was observed for *EN2* that was dependent upon both the A-C haplotype and affection status ($P<.001$). These data are consistent with increased levels of *EN2* contributing to ASD risk. To investigate when and where this happens during brain development, we generated transgenic mice. The transgene includes ~20kb of evolutionarily conserved cis-regulatory sequence, and human *EN2* is replaced with a reporter, Ds-Red. 6 A-C (ASD associated haplotype) and 8 G-T (unassociated haplotype) lines were established and characterized. QRTPCR analysis was performed on all lines at 5 developmental time points that are

representative of different stages of *En2* expression and function (E9.5, E12.5, E17.5, P6 and adult). The A-C haplotype results in increased expression at all time points ($P < .001$).

To evaluate whether this increased expression was due elevated levels in the same cell types or expanded expression domains, *in situ* hybridizations were performed. At E9.5, E17.5 and adult expanded expression domains are observed in A-C transgenic lines

We have also initiated two collaborations. The first is with Manny DiCicco-Bloom MD (UMDNJ-Robert Wood Johnson Medical School), who has demonstrated that the *En2* knockout affects the development of serotonin and norepinephrine neurons. The second is with Jacki Crawley PhD (NIH), who has investigated if the *En2* knockout displays behavioral phenotypes relevant to ASD.

Significance of the research to the field of autism:

The significance of our research is:

- Our data provides evidence that A-C haplotype is functional and results in increased expression in cell culture, *in vivo* in transgenic mice and in human post-mortem samples.
- These data are consistent with increased expression of *EN2* contributing to ASD risk.
- The transgenic results have identified the cell types and ages when the A-C haplotype is functional during development
- Research with our collaborators has demonstrated that *En2* regulates developmental processes relevant to ASD

How the findings advance the field of autism:

Our data provide additional evidence that *EN2* is a susceptibility gene as well as when, where and how the ASD-associated haplotype contributes to ASD risk.

The future directions for this line of research:

Numerous CpG islands flank human *EN2*, suggesting that differential methylation may affect expression levels. We are now investigating whether epigenetic differences contribute to increased *EN2* levels in the post-mortem samples.

To determine how increased levels of *EN2* affect down-stream molecular and cell biological pathways, several mouse models are being generated.

Xue Ming, M.D., Ph.D.

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Principal Investigator: Xue Ming, M.D., Ph.D.

Title: Neurotoxicants- Induced Behavioral Impairment in Engrailed Mutant Mice.

Project Period: 7/05-6/07

Abstract:

Autism spectrum disorder (ASD) is a prevalent and inheritable neurodevelopmental disorder. Recent human genetic studies are consistent with the homeobox transcription factor, ENGRAILED 2 (EN2), being an ASD susceptibility gene. En2 knockout mice (En2(-/-)) display subtle cerebellar neuropathological changes similar to what has been observed in the ASD brain. To investigate whether En2(-/-) mice displayed abnormal behavior relevant to ASD, they were monitored in tasks designed to assess social maturation as well as learning and memory. Deficits in social behavior were detected in En2(-/-) mice across maturation that included decreased play, reduced social sniffing and allogrooming, and less aggressive behavior. Deficits in two spatial learning and memory tasks were also observed. Because locomotor activity was a component of many of the behavioral tasks, this was measured at various stages of development. Locomotor activity was not compromised in the knockout. However, a more thorough analysis of motor behavior in En2(-/-) mice revealed deficits in specific motor tasks. To determine whether neurochemical changes were associated with these behavioral phenotypes, monoamine levels in specific brain regions were assessed. A cerebellar-specific increase in serotonin and its metabolite was observed. Interestingly, several reports have suggested that the serotonin pathway is affected in ASD. We conclude that En2(-/-) mice display behavioral and neurochemical changes, in addition to genetic and neuropathological changes, relevant to ASD. Therefore, these mice may be useful as an animal model of autism.

Highlights of the research (past and present): Environmental toxins and genetic susceptibility in autism and animal models of autism, oxidative stress in autism, clinical comorbidity of autism.

Publications and Presentations:

Chen MA, Millonig JH, **Ming X**, Roselli LM, Wagner GC (2006). Behavioral Development of the En2(-/-) Mouse: Relevance to Autistic Disorder. *Brain Research*. Oct 20;1116(1):166-76.

Ming X, Cheh MA, Yochum CL, Halladay AK and Wagner GC. (2008). Evidence of Oxidative Stress in Autism Derived From Animal Models. *American Journal of Biochemistry and Biotechnology*. 4(2): 218-225.

Yochum CL, Dowling P, Reuhl KR, Wagner GC, **Ming X**. (2008). VPA-induced apoptosis and behavioral deficits in neonatal mice. *Brain Res*. Apr 8;1203:126-32.

Research activities:

Engrailed gene polymorphism is associated with autism. We compared acquisition and regression of developmental, cognitive and behavioral skills in wild type and engrailed knockout mice. Engrailed knock-out mice showed delayed in acquisition of multiple motor, cognitive and social tasks, in comparison to wild type mice. In addition, engrailed knock-out mice showed neurochemical characteristics resembling human autism.

Significance of the research to the field of autism:

This mouse model is relevant and useful for autism research.

How the findings advance the field of autism:

Animal model is essential in test research hypothesis, prevention and treatment of autism.

The future directions for this line of research:

Future direction includes testing environmental toxins in this engrailed knock-out mice to test the hypothesis that gene by environment interaction contributes to autism pathogenesis.

Other Support:

DOD AS073084: Developing treatment, treatment validation and treatment scope in the setting of an autism clinical trial. Program project. PI William S. Johnson. 10/08 – 09/11, \$2,349,528 direct costs. 20% effort.

Robert G. Nagele, PhD

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Email address: nagelero@umdnj.edu**Principal Investigator:** Robert G. Nagele, PhD**Title:** Autoantibodies and the Pathogenesis of Autism**Project Period:** 2005-2008

Abstract: Numerous studies have shown the involvement of autoimmunity in a variety of neurological disorders, including Tourette syndrome, obsessive compulsive disease, pediatric autoimmune neuropsychiatric disorders associated with streptococcus (PANDAS) and Alzheimer's disease. Brain-specific autoantibodies have recently been isolated from the serum of autistic children, raising the possibility that binding of anti-neuronal autoantibodies to brain neurons is involved in the pathogenesis of autism. To investigate this possibility, we have developed a research project with three major experimental aims. First, we will analyze serum derived from autistic patients in an effort to determine the number and titer of autoantibodies that bind to neurons in the cerebellum, cerebral cortex, hippocampus and amygdala of normal and autistic brains using a combination of immunohistochemistry and western analysis of proteins derived from human brain membrane protein fraction. Cell type-, brain region- and patient-specific differences in autoantibody target distribution will be compared among and between autistic and control brains. Second, neuron-binding autoantibodies will be isolated directly from serum and autistic brain tissue using biochemical methods, including protein A/G columns,. Abundant protein targets of interest will be identified using immunoprecipitation, Western analysis and MALDI-mass spectrometry. Third, we will determine how living (cultured) brain neurons respond to the binding of autoantibodies derived from serum of autism patients, with emphasis on two features of neurons relevant to the development of autism: (1) their ability to grow new processes (axons and dendrites) and (2) their ability to maintain established interconnections, including synaptic contacts and their spatial relationship with surrounding glial cells. Our hope is that these studies will shed new light on the cellular basis for the pathogenesis of autism.

Highlights of the research (past and present):

Demonstrated the key role of blood-brain barrier breakdown and brain-reactive autoantibodies in the initiation and progression of Alzheimer's and Parkinson's diseases.

-Provided evidence that the presence of specific brain-reactive autoantibodies in the blood can both trigger and contribute to the progression of Alzheimer's disease and most likely other neurodegenerative diseases in the context of blood-brain barrier breakdown

-Provided evidence in a mouse model that the blood-brain barrier becomes impermeable to blood immunoglobulins by gestation day 18, immediately preceding lamination of neurons and a surge in the wiring of the cerebral cortex.

-Used the presence of these autoantibodies to develop simple blood tests for the diagnosis of Alzheimer's disease and Parkinson's disease with unprecedented accuracy

-Founded a start-up company (During Technologies, Inc) to develop these diagnostics

- Publications and Presentations:

This funding has led to the publication of a number of manuscripts demonstrating the key role of blood-brain barrier breakdown and brain-reactive autoantibodies in the pathogenesis of a number of neurodegenerative diseases. We are pursuing a similar scenario for the initiation of autism.

Publications:

1. Siu G, P Clifford, M Kosciuk, V Venkataraman and RG Nagele (2006) Glial cells and Abeta peptides in Alzheimer's disease pathogenesis. In : Abeta Peptides and Alzheimer's Disease (CJ Barrow and DH Small, eds.), Springer, pp.216-233
2. Clifford PM, Zarrabi S, Siu G, Kinsler KJ, Kosciuk MC, Venkataraman V, D'Andrea MR, Dinsmore S, and Nagele RG. (2007) Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons, Brain Research, 1142: 223-236.
3. Clifford PM, Siu G, Kosciuk MC, Levin EC, Venkataraman V, D'Andrea MR, and Nagele RG. (2008) $\alpha 7$ nicotinic acetylcholine receptor expression by vascular smooth muscle cells facilitates the deposition of A β peptides and promotes cerebrovascular amyloid angiopathy. Brain Research, 1234:158-171.
4. Levin E, Acharya NK, Sedeyn JC, Venkataraman V, D'Andrea MR, Wang H-Y, and Nagele RG. (2009) Neurons express vimentin in Alzheimer's disease brain as part of a generalized dendritic damage-response mechanism. Brain Research, 1298:194-207
5. Levin, EC, NK Acharya, M Han, S Zavareh, JC Sedeyn, V Venkataraman and RG Nagele. (2010) Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathological changes in the context of blood-brain barrier breakdown. Brain Research, 1345(23):221-232.

6. Nagele RG, Clifford PM, Siu G, Levin EC, Acharya NK, Han M, Kosciuk MC, Venkataraman V, Zavareh S, Zarrabi S, Kinsler K, Patel N, Nagele EP, Dash J, Wang HY, Levitas A (2011) Brain-Reactive Autoantibodies Prevalent in Human Sera Increase Intraneuronal Amyloid- β 1-42 Deposition. *Journal of Alzheimer's Disease* 25: 605-622.

Other support: The UMDNJ Foundation Venture Capital Group, GlaxoSmithKline and the Michael J Fox Foundation.

Research activities: See relevant articles listed above.

Significance of the research to the field of autism:

The research funded here has led us to hypothesize that the combination of the presence of specific brain-reactive autoantibodies in the blood that react specifically with the surfaces of brain neurons plus an environment-induced developmental delay in “closure “ of the blood-brain barrier could collaborate to trigger autism in the infant. If true, then the specific autism spectrum phenotype may be entirely dictated by the presence, location and extent of a developmental delay in blood-brain barrier closure in the infant, the presence of specific neuron-binding autoantibodies in the infant serum and the relative abundance of the neuronal targets of these autoantibodies. Further, since autoantibodies in the infant during the first few month of life are acquired from the mother’s blood, this suggests that it is possible to detect specific autoantibodies in the serum of the mother (and hence the newborn) that would predispose the infant to autism under environmental conditions favoring a developmental delay in closure of the blood-brain barrier. We are currently trying to address this question in collaborative study using human protein microarrays with Dr. Xue Ming.

How the findings advance the field of autism: This work has advanced the field of autism by leading to a new hypothesis that provides a reasonable explanation for the dramatically increased incidence of this disease in the last 30 years or so.

The future directions for this line of research: We are now ready to use our newly developed disease diagnostic platform to screen the blood of autistic children and their mothers for the presence of autism-specific autoantibodies that may play a role in both predisposing certain individuals for autism and triggering this devastating disorder.

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Principal Investigator: Nicholas M. Ponzio, Ph.D.

Title: Influence of immune mechanisms on the development of autism spectrum disorders

Project Period: July 1, 2005 – June 30, 2007

Abstract:

Autism spectrum disorder (ASD) is a perinatal developmental disorder characterized by behavioral, neurological, and immunological abnormalities. ASD is a multifactorial disorder, the causes of which have not been fully defined. However, clinical and experimental studies indicate that in addition to genetic predisposition, immune mechanisms, in general, and cytokine dysregulation, in particular, are contributing etiological factors in ASD. In murine models of autism, it has been shown that immune stimulation of pregnant dams with cytokines (IL-2, IL-6) or polyclonal activators (poly I:C, LPS) at mid-gestation causes autism-like behavioral abnormalities in their offspring in comparison to offspring of control PBS-injected dams. We examined these murine prenatal models of autism to determine if immunostimulation during pregnancy also causes alterations in the development and/or function of lymphoid and myeloid lineages in offspring, with particular emphasis on analysis of T helper (TH) subsets. In the first model, we administered five daily i.p. injections of murine IL-2 to pregnant dams during mid-gestation (E12-E16). In second model, pregnant dams were given one i.p. injection of poly I:C (pl:C) at E12. Spleen cells from offspring of immunostimulated dams (IL-2 and pl:C pups) were compared to offspring of pregnant mice injected with vehicle only (PBS pups) for mixed lymphocyte reaction (MLR) and cytotoxic T lymphocyte (CTL) function, and cytokine production. Multi-color flow cytometry (FACS) analysis and TH cell differentiation cultures were also performed on spleen cells from pl:C and PBS pups. Sera and amniotic fluids from pregnant dams and supernatants from activated lymphocyte cultures of offspring were analyzed for the presence of multiple cytokines by Luminex assay. In these well-characterized prenatal mouse models of autism, we observed high levels of pro-inflammatory cytokines in maternal sera and amniotic fluids, and changes in the T cell subsets of their offspring. In the model where pregnant dams were injected with IL-2 (vs. PBS), in addition to their previously shown abnormal

behavior, IL-2 pups also exhibited accelerated T cell development, with a skewing toward TH1 cell differentiation, and significantly higher MLR and CTL responses. In the second model, 2-16 hrs after injection of pregnant dams with pl:C (vs. PBS), high levels of pro-inflammatory cytokines were detected in their sera and amniotic fluids. In addition, FACS analysis of activated T cells from neonatal and adult offspring of pl:C-injected pregnant dams showed higher percentages of CD4⁺ TH cells with intracellular IL-17 (TH17 cells) than neonates from PBS-injected dams. These results demonstrate that maternal immune activation during pregnancy caused production of cytokines that promoted the development of a **pro-inflammatory phenotype** in offspring, and the development of TH1 and TH17 cells in their peripheral lymphoid tissues. These effects were already present in 2-3 week old neonates, and persisted into adulthood. Since TH1 and TH17 cells have each been shown to mediate pathogenesis in other mouse models of human diseases, their presence in the offspring of IL-2 and pl:C-injected pregnant dams suggests that TH1 and TH17 cells may contribute to the immunological and behavioral abnormalities observed in these prenatal rodent models of autism.

Highlights of the research (past and present):

A major focus of this research project was to refine an existing prenatal mouse model of ASD to investigate immunological outcomes in offspring of pregnant dams that are given immune stimulation. The subsequent research that we pursued was to determine the effects of cytokine exposure *in utero* on the immunological profile of offspring. Initially, pregnant dams were injected with IL-2, however, over the course of the investigation pregnant dams were also given immune stimuli other than IL-2 (such as pl:C), and the offspring of these immune stimulated dams were analyzed in comparison to offspring of PBS-injected dams. Offspring of immune-stimulated pregnant dams showed accelerated T cell development, as judged by functional assays for proliferation in response to immune stimulation, cytokine production, and cytotoxic activity. The pattern of responses seen in these offspring is consistent with involvement of pro-inflammatory phenotype and the development of a specific T Helper (TH) cell subset known as TH17 cells. Spleen cells from the offspring of pl:C (vs. PBS) injected pregnant dams were cultured *in vitro* with polyclonal activators, and the resulting cell populations were subjected to FACS analysis to determine their phenotype. The results showed a significantly higher frequency of **pro-inflammatory TH17 cells** in offspring obtained from pl:C-injected (vs. control PBS-injected) dams.

Given the fact that TH17 cells have been shown to mediate pathogenesis in other disease models (e.g., Multiple Sclerosis, Rheumatoid Arthritis, and Inflammatory Bowel Disease), the significance of these findings is compelling. The model in which pl:C is injected to pregnant dams during mid-gestation has been validated in the literature as an autism model, and has

been shown to cause behavioral abnormalities in their offspring (in comparison to offspring of pregnant dams injected with PBS. Our results demonstrate that these offspring also exhibit a pro-inflammatory phenotype, which may contribute to the immunologic dysregulation in autism.

Resulting publications and presentations:

a. Publications in peer-reviewed journals

Ponzio NM, Servatius R, Beck K, Marzouk A, Kreider T: Cytokine levels during pregnancy influence immunological profiles and neurobehavioral patterns of the offspring. *Ann NY Acad Sci.* 1107:118-128, 2007.

Mandal M, Marzouk AC, Donnelly R, Ponzio, NM: Preferential development of TH17 cells in offspring of immunostimulated pregnant dams. *J Reproductive Immunol.* 87:97-100, 2010.

Mandal M, Marzouk AC, Donnelly R, Ponzio NM: Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells. *Brain, Behavior, and Immunity.* 2011 (*In Press*)

Zalcman SS, Bobbin M, Bishayee S, Marzouk A, Rossi-George A, Ponzio NM: Maternal exposure to IL-2 induces long-lasting increases in behavioral and neuronal responses to novelty and psychostimulant challenge and T cell function in offspring. (*In Preparation*)

Mandal M, Donnelly R, Elkabes S, Ponzio NM: Maternal immune stimulation during pregnancy facilitates prenatal immuno-developmental changes leading to a pro-inflammatory phenotype in offspring. (*In Preparation*)

Mandal M, Donnelly R, Zhang P, Ponzio NM: Fetal programming in offspring of dams that receive immune stimulation during pregnancy promotes exaggerated acute inflammatory cytokine and cellular responses to a Toll-Like Receptor agonist. (*In Preparation*)

b. Presentations at Scientific Meetings

Ponzio NM, Jonakait GM, Mandal M, Pratt L, Marzouk AC, Yehia G: Immunostimulation during pregnancy alters the development of T helper subsets in mother and offspring in a prenatal model of autism. 6th International Congress on Autoimmunity. Porto, Portugal. September 10-14, 2008.

Mandal M, Marzouk AC, Yehia G, Ponzio, NM: Immunostimulation during pregnancy alters development of T helper cell subsets of offspring in prenatal models of autism. Annual meeting of the Society for Neuroscience. Washington, DC. November 15-19, 2008.

Mandal M, Marzouk A, Donnelly R, Yehia G, and Ponzio, NM. Maternal immune activation

during pregnancy alters development of T helper cell subsets of offspring in prenatal models of autism. 8th Annual International Meeting for Autism Research. Chicago, IL. May 6-8, 2009.

Ramanathan M, Ponzio NM and Fernandes H: Maternal cytokine regulation in the pathogenesis of autism. 5th Asian Pacific Congress in Maternal Fetal Medicine. November 2009; Hong Kong, China.

Pratt L, Ponzio NM, Ni L, Sheng I, and Jonakait GM: Fetal microglia become activated following maternal immune challenge. 9th Annual International Meeting for Autism Research. Philadelphia, PA. May 20-22, 2010.

Mandal M, Marzouk AC, Donnelly R, Ponzio NM: Preferential differentiation of TH17 cells in offspring of immune-activated dams in a prenatal mouse model of autism. 9th Annual International Meeting for Autism Research. Philadelphia, PA. May 20-22, 2010.

Mandal M, Marzouk AC, Donnelly R, Ponzio NM: Preferential differentiation of TH17 cells in offspring of immune-activated dams in a prenatal mouse model of autism. Annual meeting of the Psychoneuroimmunology Research Society. Dublin, Ireland. June 2-5, 2010.

Ramanathan M, Ponzio NM, Limson F, Shah S, and Fernandes H: Maternal cytokine regulation in the pathogenesis of autism. 9th Annual International Meeting for Autism Research. Philadelphia, PA. May 20-22, 2010.

Agley AL, Jiao X, Beck KD, Pang KCH, Ponzio NM, Servatius RJ: A diathesis approach to modeling autism in rats: Interaction of immune activation in dams of either normal or inhibited temperament. Society for Neuroscience Annual Meeting satellite event: Emerging neuroscience of autism spectrum disorders.. November 11-12, 2010. San Diego, CA.

Mandal M, Marzouk AC, Donnelly R, Ponzio NM: In utero cytokine exposure influences postnatal development of T helper cells. 10th Annual International Meeting for Autism Research. San Diego, CA. May 12-14, 2011.

Mandal M, Basak S, Marzouk AC, Donnelly R, Ponzio NM: Pre-natal cytokine exposure influences postnatal development of T helper cells. 18th Annual meeting of the Psychoneuroimmunology Research Society. Chicago, IL. June 8-11, 2011.

Mandal M, Donnelly R, Elkabes S, Ponzio NM: Maternal immune stimulation during pregnancy facilitates prenatal immuno-developmental changes leading to a pro-inflammatory phenotype in offspring. 11th Annual International Meeting for Autism Research. Toronto, Ontario. May 17-19, 2012.

Other Support:

Title: Influence of the maternal immune response on the development of autism

Source: Autism Speaks Period: 07/01/2008 – 12/31/2011

Total Direct Costs: \$450,000

Title: Influence of the maternal cytokines on activation of the innate immune system as a factor in the development of autism

Source: Autism Speaks (Fellowship for M Mandal) Period: 06/01/2008 – 05/31/2010

Total Direct Costs: \$64,000

Title: New rodent models of autism.

Source: Foundation of UMDNJ & NJMS Dean's Biomedical Research Support Program

Period: 01/01/2009 – 06/30/2010 Total Direct Costs: \$25,000

Title: Influence of the maternal immune system on development of autism

Source: Autism Speaks Period: 04/01/2009 – 03/31/2012

Total Direct Costs: \$300,000

Significance of the research to the field of autism:

As a result of this grant award, we developed a new experimental model and new research direction to better understand and explain the basis for the co-morbidity of immune dysregulation in a significant cohort of children with autism.

How the findings advance the field of autism:

Results from the mouse studies initiated by this grant also gave rise to a translational research project to investigate Single Nucleotide Polymorphisms (SNPs) in regulatory regions of cytokine genes from mothers of children with ASD. Our preliminary results correlate nicely with the data that we have obtained from the prenatal mouse model that we have been studying. These results demonstrate that mothers of autistic children who also exhibit some form of immune dysregulation have a greater frequency of SNPs that promote elevated production of some of the same pro-inflammatory cytokines we have observed in the mouse model.

The future directions for this line of research:

In future research, we plan to determine the mechanisms for development of the pro-inflammatory phenotype in offspring of pregnant mice that receive immunostimulation. We feel that these future studies will give additional direction to our translational research project, and provide a foundation to consider interventional therapeutic strategies to neutralize or inhibit maternal immune responses during pregnancy that result in development of a proinflammatory phenotype in offspring.

Gleb Shumyatsky, Ph.D.

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Principal Investigator: Gleb Shumyatsky, Ph.D.

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

Project Period: 2010 - 2012

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 enriched in the basolateral amygdala (BLA) will be examined. Hypothesis: perturbations in amygdala-enriched genes are critical for social and emotional states related to the ASD behavioral abnormalities. In Aim 1, different types of social and emotional behaviors related to ASD will be probed as being controlled by amygdala-enriched genes. The three core ASD features are abnormalities in social behavior, communication, and inflexibility/repetitive behaviors. Two knockout lines for amygdala-enriched genes, gastrin-releasing peptide receptor (GRPR) and zinc transporter 3 (ZnT3) 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. In Aim 2, 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 GRP gene promoter which drives transgene expression in the amygdala neural circuitry and the reversible tTA/tetO transgenic system allowing turning transgenes on and off. It will be examined at what developmental stage turning off abnormal stathmin gene expression in the BLA-associated 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. There is a possibility of reversing the phenotype at the adult stage. If the hypothesis is correct, future treatments of ASD with the focus on amygdala gene expression can be envisioned. Importantly, the GRPmtTA transgenic line is the first to drive gene expression specifically in the amygdala neural circuitry and thus holds great promise for transgenic research of normal brain function as well as its pathology.

Highlights of the research (past and present): We have found amygdala-enriched genes and characterized some of them in social behaviors related to autism. (1) One surprising finding is

that independently of anxiety state (decreased, increased or normal) social behaviors seem to be always affected by these genes, suggesting an exciting possibility of separating anxiety and social behaviors most likely at the level of the corresponding neural circuits. (2) Another very intriguing finding is that we can reverse social abnormalities in transgenic mice by turning the transgenes off at the adult stage, this in turn suggests that treatments are achievable at the adult stages of social behaviors related to ASD. This may have important implications to treatment ASD.

Publications and presentations:

Light K., Uchida S., Martel G., and Shumyatsky G.P.. Social and flexibility deficits in mouse models of ASD based on amygdala-enriched genes. Poster Presentation at the 29th Annual Autism New Jersey Conference. October 13, 2011.

Martel G, Uchida S, Takizawa S, Paulovich K, Hevi C, and GP Shumyatsky. Exploring the behavioral effects of a conditional mutation of stathmin. Rutgers Brain Health Institute Symposium on Autism and Alzheimer's disease. October 25, 2011.

Martel, G., Hevi, C., Friebely, O., Baybutt, T., and Shumyatsky, G.P. (2010). Zinc transporter 3 is involved in learned fear and extinction, but not in innate fear. *Learn Mem* 17, 582-590.

Martel, G., Hevi, C., Kane-Goldsmith, N., and Shumyatsky, G.P. (2011). Zinc transporter ZnT3 is involved in memory dependent on the hippocampus and perirhinal cortex. *Behav Brain Res* 223, 233-238.

The results of this work were also presented at (1) the Gordon Conference "Amygdala in Health and Disease", August 2011 (Colby College, Maine), (2) Molecular and Cellular Cognition Society, November 2011 (Washington, DC) and the Annual Meeting of the Society for Neuroscience, November 2011 (Washington, DC).

Martel G., Hevi C., Wong A., Zushida K., Uchida S., & Shumyatsky G.P. (2012) GRPR and stathmin control in opposite directions both cued fear extinction and neural activities of the amygdala and prefrontal cortex. *PLoS One*. 7(2):e30942. Epub 2012 Feb 1.

Other Support:

1R01 MH080328-02

Shumyatsky (PI) 08/08/09-08/07/12

The role of GRP and GRPergic circuitry in fear memory

The goal of this project is to compare the roles GRP and GRPR in fear learning

Role: PI

Whitehall Foundation

Shumyatsky (PI) 02/01/09-01/31/12

Genetic tracing and functional analysis of amygdala microcircuits

The goal of this project is to understand the role of microcircuits in the amygdala

Role: PI

NJ Governor's Council on Autism

Shumyatsky (PI) 06/01/10-05/31/12

Amygdala-enriched genes in behaviors associated with ASD

The goal of this project is to understand the role of stathmin in social and fear behaviors related to autism

Role: PI

Research activities:

Our lab is involved in studying the molecular mechanisms that operate on the amygdala-associated neural circuits controlling memory of fear, innate fear, social and maternal behaviors.

Significance of the research to the field of autism:

Our research is aimed to provide insights into the molecular basis of the intrinsic neuroanatomic connections within the amygdala and hippocampus that underlie fear-related, anxiety and social behaviors that are deficient in patients with ASD.

How the findings advance the field of autism:

Our research provides genetic and anatomic evidence the amygdala controls social behaviors via two pathways. The first pathway is directly originates from the amygdala, by controlling innate fear and anxiety. The second pathway is by amygdala anatomic connections with the cortex and hippocampus; in this case innate fear and anxiety do not have to be disturb to control social behaviors.

The future directions for this line of research:

By rescuing social behavior abnormalities in the juvenile and adult mice, we show that these social phenotypes can be reversed. Thus, medical treatments can be envisioned that work by changing protein function at any developmental stage in humans which can be based on the biochemical signaling pathways that we study.

Samuel S.-H. Wang, Ph.D.

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Principal Investigator: Samuel S.-H. Wang, Ph.D.

Title: "Sensory Processing in the Cerebellum of Awake Animals."

Project Period: 2005-2007

Abstract: During the grant period, the laboratory implemented methods for in vivo multiphoton optical imaging of identified groups of neurons in the cerebellum of anesthetized and awake mice. Complex spikes were imaged in Purkinje cells, which are reduced in number in autism. In wild-type mice, Purkinje cell responses were imaged in response to external sensory stimuli and to internally generated programs for movement. A key feature was synchrony across populations of Purkinje cells, which may encode unexpected events. These results establish a baseline for investigating model systems such as SHANK3 and CNTNAP2 mutant mice, which express behavioral features reminiscent of autism. Aberrations in synchronous firing would be consistent with a loss of function in conveying unexpected events to the rest of the brain. In the future, a deeper understanding of cerebellar dysfunction in autism may inspire early-childhood interventions to compensate for lost cerebellar function. The hypothesis to be tested in the future is that the cerebellum plays an essential guiding role during critical periods for social development, both in neurotypical and autistic brains.

Highlights of the research (past and present): My laboratory is primarily concerned with basic research on mechanisms of learning, memory, and other forms of brain plasticity.

IMAGING THE CEREBELLUM DURING LEARNING. We use advanced optical methods to probe the basic function of the cerebellum, whose gross proportions and cell numbers are very frequently affected in autistic persons. The cerebellum, a processor of unexpected events, critically influences action, cognition, and social/emotional processing. We use two-photon microscopy to image cerebellar activity in awake mice.

SYNAPTIC LEARNING RULES: In addition, in past years we have identified fundamental principles by which molecular signaling mechanisms shape learning rules. For example, we have found that calcium signaling mechanisms drive the switchlike strengthening and weakening of single

synapses. The likelihood and direction of this change is closely dependent on the precise occurrence of certain presynaptic and postsynaptic spike patterns.

Publications and Presentations:

2002- NIH R01 NS045193: Synaptic learning rules in the mammalian cerebellum
2008-2010 Autism Speaks postdoctoral support for Ilker Ozden, Ph.D.
2009-2011 NIH Challenge Grant (Co-investigator; PI Lynn Enquist)
2009-2011 NIH Challenge Grant (Co-investigator; PI David Tank)

Selected relevant publications:

M.R. Sullivan, A. Nimmerjahn, D.V. Sarkisov, F. Helmchen, and S.S.-H. Wang (2005) In vivo calcium imaging of circuit activity in cerebellar cortex. *Journal of Neurophysiology*, 94:1635-1643. *Dr. Sullivan was a recipient of a National Alliance for Autism Research predoctoral training grant.*

I. Ozden*, H.M. Lee*, M.R. Sullivan, and S.S.-H. Wang (2008) Identification and clustering of event patterns from in vivo multiphoton optical recordings of neuronal ensembles. *Journal of Neurophysiology*, 100:495-503. *Dr. Ozden was a recipient of an Autism Speaks postdoctoral fellowship.*

T.M. Hoogland, B. Kuhn, W. Göbel, W. Huang, J. Nakai, F. Helmchen, S.J. Flint, and S.S.-H. Wang (2009) Radially expanding transglial calcium waves in the intact cerebellum. *Proc. Natl. Acad. Sci. USA*, 106:3496-3501.

I. Ozden, M.R. Sullivan, H.M. Lee, and S.S.-H. Wang (2009) Reliable coding emerges from coactivation of climbing fibers in microbands of cerebellar Purkinje neurons. *Journal of Neuroscience*, 29:10463-10473.

A.E. Granstedt, M.L. Szpara, B. Kuhn, S.S.-H. Wang, and L.W. Enquist (2009) Fluorescence-based monitoring of activity in virally traced neural circuits. *PLoS ONE*, 9:e6923.

A.E. Granstedt, B. Kuhn, S.S.-H. Wang, and L.W. Enquist (2010) Calcium imaging of neuronal circuits in vivo using a circuit-tracing pseudorabies virus. *Cold Spring Harbor Protocols*, 2010(4):pdb.prot5410.

I. Ozden, D.A. Dombeck, T.M. Hoogland, D.W. Tank, and S.S.-H. Wang. Widespread state-dependent shifts in cerebellar activity in locomoting mice. In review.

B.C. Campbell and S.S.-H. Wang (2012) Familial linkage between neuropsychiatric disorders and intellectual interests. PLoS ONE, 7(1):e30405. doi:10.1371/journal.pone.0030405 (#4 most-viewed in 30 days)

Sam Wang: Autism myth lives on. USA Today, April 16, 2008. An op-ed on the vaccine/autism myth.

Other Support:

Simons Foundation Autism Research Initiative (SFARI) exploratory research grant to screen mouse autism lines (for example, SHANK3, CNTNAP2) for cerebellar functional deficits.

Significance of the research to the field of autism:

Recent research has illuminated a broad role for cerebellum as a general processor of unexpected events. In autistic persons, defects are most commonly seen in the vermis, a midline cerebellar structure thought to serve cognitive and affective functions. The Wang lab is among the first in the world to make extensive use of multiphoton optical methods to probe what cerebellar circuits do during awake behavior. One central finding arising from the supported work finds that information is encoded in the synchronous complex spiking of many neighboring Purkinje cells, which provide the cerebellum's output.

How the findings advance the field of autism:

Synchronous spiking is likely to encode unexpected events arising from external events. Such processing may be aberrant in autistic persons, who have notable sensory sensitivities. The cerebellum is also a putative site for multimodal integration. Two-year-olds with autism have difficulty with one multimodal task, the matching of visual biological motion with appropriate spoken words (A Klin et al., Nature). Thus aberrant multisensory integration is an early feature in the development of autistic brains. Understanding the cerebellar substrates of such integration may provide an opportunity for translational research to remedy such deficits in early childhood.

The future directions for this line of research:

In the coming years, we wish to test the hypothesis that cerebellar abnormality in early life acts as a developmental trigger for autism. Just as Hubel and Wiesel demonstrated that visual input is needed to establish a normal visual cortex, internal brain activity might also guide maturation. We term this the *developmental diaschisis hypothesis*. This term specifically denotes effects that accumulate during a sensitive period, as opposed to the usual diaschisis concept, in which function is lost immediately after damage to a distant region. A developmental role for cerebellum is consistent with three observations: (1) Pediatric insult to

the vermis leads to cognitive and affective deficits. (2) Cerebellar injury associated with premature birth is followed by autism-like symptoms and reduced prefrontal volume at age two. (3) In rats, midline cerebellar lesions at P10 cause perseveration and social disruption in the adult.

In one set of experiments, we will test if reversible inactivation of cerebellum during development disrupts adult function. We will inactivate rodent cerebellum using molecular tools for neural silencing at P9-15, corresponding approximately to human third trimester. Adult animals will then be tested for perturbations in (a) a 3-chamber social interaction test, (b) ultrasonic vocalization, and (c) coding of sensory events in the cerebellum, (d) eyeblink conditioning. The developmental diaschisis hypothesis predicts changes in (a) and (b) but not necessarily (c) or (d), and further predicts that changes will depend critically on the specific dates of inactivation.

Harvey R. Weiss, Ph.D.

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Principal Investigator: Harvey R. Weiss, Ph.D.

Title: Autism and control of cerebral metabolism

Project Period: 2005 - 2007

Lay Abstract: 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.

Highlights of the research (past and present): We have examined the brains of a rat model of tuberous sclerosis as our model of autism spectrum disorders. Most children with tuberous sclerosis develop autism spectrum disorders. We have found significantly increased cerebral metabolism. This suggests that increased rather than decreased brain activity may be associated with autism spectrum disorders. We have now established that this increased brain metabolic activity is not related to increased activity of the glutamate excitatory amino acid neurotransmitter system.

Publications and Presentations: We have submitted two manuscripts for publication. Two additional studies are nearing completion. A third manuscript is now in preparation.

Other Support: A grant application has been submitted to the NIH using our animal model of autism spectrum disorder.

Research activities: Autism spectrum disorders (ASD) can lead to changes in both excitatory and inhibitory neurotransmitter systems. We are studying these changes in an animal model of tuberous sclerosis and ASD (the Eker rat). Our preliminary data indicate dramatic changes in cerebral oxygen consumption and how that oxygen consumption is controlled. These changes occur in young animals at a time when ASD is first noticed. We have also found a reduced basal level of activity in a part of the glutamate excitatory neurotransmitter system (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors) in the control of brain metabolism.

We have, therefore, measured cerebral oxygen consumption after stimulation of this system to determine if its metabolic effects were reduced. We tested the hypothesis that increased stimulation of AMPA receptors would not augment cerebral O₂ consumption in the Eker rat. Three cortical sites were used for administration of saline and two doses of AMPA in young (4 weeks) male control Long Evans and Eker rats. Cerebral O₂ consumption was determined in isoflurane anesthetized rats. Receptor levels were also studied. We found significantly increased cortical O₂ consumption (+33%) with AMPA, Fig. 1. Cortical AMPA receptor protein levels were significantly reduced (-21%), Fig. 2. These data demonstrated a reduced importance of AMPA receptors in the control of cortical metabolism, related to reduced AMPA receptor protein, in the Eker rat. This suggests that increasing AMPA receptor

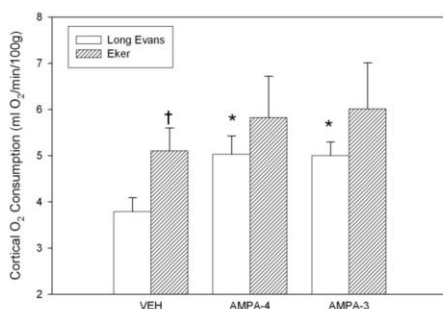


Figure 1: Cortical O₂ consumption in control (unhatched bars) and Eker (hatched bars) rats. The regions shown were vehicle treated or treated with 10⁻⁴ or 10⁻³ M AMPA. In control, but not Eker rats, AMPA increased cortical O₂ consumption. Eker rats had significantly higher cortical O₂ consumption than Long Evans rats in the vehicle treated region. Values are mean ± S.E.M. * indicates a value different from vehicle. † indicates a value different from corresponding region in control rats.

activity may not be an effective treatment for children with autism spectrum disorders as they also have reduced AMPA receptor number.

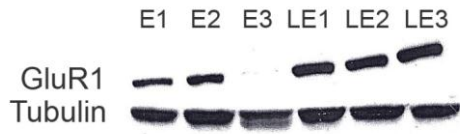


Figure 2: Protein levels of the AMPA receptor GluR1 subunit probed with a specific antibody measured by Western blotting. Tubulin was used as the control. The cortexes from three Eker and three Long Evans (control) rats are shown. The protein levels of the AMPA receptor subunit were lower in the Eker rats compared to the Long Evans.

We are now trying to determine why this model has a defect in a gene that suppresses the action of mTOR (mammalian target of rapamycin). When active, mTOR can lead to tumor growth. We are testing rapamycin's effect on cerebral metabolism in this model compared to control animals. Preliminary data indicate that its effect in decreasing O_2 consumption is much greater in the Eker rat compared to controls. There have been suggestions that rapamycin might be useful in ASD. Our model appears to closely resemble, at least, some children with ASD. This suggests that our findings may eventually translate into new treatments for ASD.

Significance of the research to the field of autism: The current studies suggest that excitatory ionotropic glutamate receptors would not be attractive targets for treatment of children with ASD. For those children with ASD that have elevated brain metabolism, inhibition may prove much more useful.

How the findings advance the field of autism: These studies suggest that inhibition of brain activity may prove to be a useful treatment for ASD. Most people always assumed that ASD was related to reduced brain activity. However, our data suggest that brain metabolism may be greatly increased.

The future directions for this line of research: We are going to study the effects of the inhibitory gamma-aminobutyric acid (GABA) neurotransmitter system. Preliminary data suggest that its role is reduced in our model. Therefore, activation of this system may prove to be of benefit in reducing the elevated cerebral metabolism associated with this model of ASD.

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Principal Investigator: Walter Zahorodny, Ph.D.

Title: Population-based surveillance of Autism Spectrum Disorder (ASD) in NJ Young-Adults with Autism

Project Period: 2007-2009

Aims:

While the descriptive epidemiology of autism in children is finally being developed, almost no attention has been directed to establishing the prevalence of autism among young adults or to describing the expression and distribution of this disorder in this growing population. The aims of this pilot study, sponsored by the New Jersey Governor's Council for Medical Research and Treatment of Autism were: 1) determination of the population-based prevalence of Autism Spectrum Disorders (ASD) in eighteen-year old adults residing in Union County, New Jersey, in 2006, using a multiple-source case review methodology, 2) objective specification of the demographic and functional characteristics of this cohort and 3) analysis of the study findings to enhance scientific understanding of autism in young adults.

Highlights of the research (past and present): The highlight of our research is the understanding that ASD prevalence is higher in NJ than in other US regions and confirmation of a significant increase in ASD prevalence between 2002 and 2006. These findings underscore the scale of ASD and suggest the interaction of genetic and environmental factors in the etiology of autism

Publications and Presentations: Thanks to Council funding, the investigator received support from CDC to implement ASD Surveillance in Union County for 2008 and, more recently, to continue ASD surveillance for 2010 in four New Jersey counties. An article describing ASD prevalence for 2006 has been submitted for publication in a peer-review Journal. Another publication on ASD prevalence in young adults is being written.

Subsequent Grant Support:

Centers for Disease Control and Prevention (CDC) Grant # DDO6-601

Enhancing Population—Based Surveillance of Autism Spectrum Disorders

Grant period: June 1, 2009 - May 31, 2010

Funding level: \$400,000

Centers for Disease Control and Prevention (CDC) Grant # 3URDD000674-01W1

Enhancing Autism Surveillance in New Jersey

Grant period: June 1, 2010 - May 31, 2011

Funding level: \$535,000

Presentations:

Autism Research in New Jersey

New Jersey Medical School - Pediatrics Department Grand Rounds, University of Medicine and Dentistry, Newark New Jersey

September 23, 2009

New Jersey Autism Study 2006: Peak Prevalence

Young Adults with Autism: Population-Based Prevalence in Union County

Centers for Disease Control and Prevention (CDC), Atlanta Georgia

Autism and Developmental Disabilities Monitoring Network Meeting,

February 22-24, 2010

Autism Surveillance: National and New Jersey Trends

Current Trends in Autism: Hear From the Experts:

Childrens' Specialized Hospital, Mountainside, New Jersey

April 23, 2010

1) Frequency of Interventions to Children with ASD in the Pre-School Period:

Findings From the New Jersey Autism Study

2) Pharmacotherapy of ASD Children in 2006: Findings From the New Jersey

Autism Study

International Meeting for Autism Research (IMFAR), Philadelphia, PA

May 22-24, 2010

New Jersey: A Bellwether of Autism Prevalence

Autism New Jersey Annual Conference, Atlantic City, New Jersey

October 21, 2010

Renping Zhou Ph.D.

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Principal Investigator: Renping Zhou Ph.D.

Title: Regulation of Axon Targeting in the Brain by Eph Family Tyrosine Kinase Receptors

Project Period: 2001-2003

Abstract: Our studies revealed that the Eph family receptors and their ligands play key roles in the wiring of the brain. These molecules guide axons to specific brain regions to form topographically accurate connections. Our studies so far demonstrated that the Eph guidance molecules contribute to the development of several neural systems, including the dopaminergic brain reward system, the hippocampal, thalamocortical, as well as corticocortical axon pathways. In addition, we have evidence that these molecules also play a role in the spinal cord development.

Highlights of the research (past and present): Formation of neural circuits and effects of disturbance of the circuits on behavior

Publications and Presentations:

1. Yue Y, Chen Z-Y, Gale NW, Blair-Flynn J, Hu T-J, Yue X, Cooper M, Crockett DP, Yancopoulos GD, Tessarollo L, and **Zhou R.** (2002) Mistargeting hippocampal axons by expression of a truncated Eph receptor. *Proc. Natl. Acad. Sci. USA* 99:10777-10782.
2. Mann F, Peuckert C, Dehner F, **Zhou R,** and Bolz J. (2002) Ephrins regulate the formation of terminal axonal arbors during the development of thalamocortical projections. *Development* 129:3945-3955.
3. Hu Z, Yue X, Yue Y, Crockett DP, Egger MD, Tessarollo L, and **Zhou R.** (2003) Corpus Callosum Deficiency in Transgenic Mice Expressing A Truncated EphA5 Receptor. *J. Neurosci.* 23: 10963-10970.
4. Chen ZY, Sun C, Bergemann A, Henkemeyer M, and **Zhou R.** (2004) Regulation of Hippocampal Axon Defasciculation by EphB Receptor Tyrosine Kinases. *J. Neurosci.* 24: 2366-2374.
5. Halladay AK, Tessarollo L, Zhou R, Wagner GC. (2004) Neurochemical and behavioral deficits consequent to expression of a dominant negative EphA5 receptor. *Brain Res Mol Brain Res.* 123:104-111.

6. Hu Z, Cooper M, Crockett DP, and **Zhou R**. (2004) Differentiation of the midbrain dopaminergic pathways during mouse development. *J. Comp. Neurol.* 476: 301-311.
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Other Support:**Current:**

PI, NIHRO1 1R01EY019012: Regulation of Lens Fiber Cells Organization. 07/01/09-06/30/14. Total Award: \$1,915,544

Project PI, NIHPO1: subproject II: Mechanisms of Neurotrophin and ephrin signal transduction. 07/01/09-06/30/14. Total award: \$1,025,311.

Completed:

PI, New Jersey Commission on Cancer Research: 7/1/2008 – 6/30/2010. Assessing Mechanisms by Which Eph Receptors Regulate Carcinogenesis. Total Award: \$120,000

PI, National Science Foundation: 03/01/06 - 02/28/09. Roles of tyrosine phosphatase in axon guidance by Eph receptors. Total Award: \$360,000.

PI, New Jersey Commission on Spinal Cord Research: 12/01/04-11/30/09, "Roles of EphA receptors and ligands in the development of the spinal cord". Total award: \$400,000.

Project PI, National Institute of Health PO-1 grant (PI: Ira Black): 4/1/03-3/31/08. My segment of the program project grant is titled "Regulation of topographic projection in the brain". Total award for my segment: \$626,875.

Co-Project PI, National Institutes of Health P-30 grant (PI: Michael Gallo): 06/01/03-05/31/08, "Core III – Neural & Developmental Toxicity." Total award for my segment: \$202,635.

Research activities:

We are currently investigating:

1. Regulation of dendritic spine and synapse formation
2. Molecular mechanisms underlying formation of brain cytoarchitecture, cell density and distribution
3. Signaling mechanisms of Eph-mediated axon guidance
4. Regulation of aggressive behavior by Eph receptors

5. Eph and circadian cycle

Significance of the research to the field of autism:

Our past and current studies reveal critical roles of Eph receptors and their ligands in establishing proper neural cytoarchitecture, pathways, and spine and synaptic densities. In addition, we now have evidence that these molecules also play important roles in behavioral regulations such as aggression and circadian cycles. These results reveal multiple functional consequences of Eph deficiencies and suggest possible involvement in autism, as evidenced by recent indications that some members of the Eph family may be implicated in autism susceptibility.

How the findings advance the field of autism:

Our mechanistic studies of Eph function during development and adult lay the foundation for future studies of roles of Ephs/ephrins in autism.

The future directions for this line of research:

We plan to continue to study:

1. Roles of Ephs/ephrins in synapse formation and function
2. Mechanisms and functions of Ephs/ephrins in autism
3. Behavior abnormalities in animals deficient in Ephs/ephrins