THE GOVERNOR'S COUNCIL FOR MEDICAL RESEARCH AND TREATMENT OF AUTISM

ANNUAL REPORT

JANUARY 1 – DECEMBER 31, 2008

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Overview

On September 12, 2007, New Jersey Governor, Jon Corzine, signed P.L. 2007, Chapter 168 into law establishing the Governor's Council for Medical Research and Treatment of Autism in the New Jersey Department of Health and Senior Services (DHSS). In moving to the Division of Family Health Services within the DHSS, the Council, which was established by State Statute in 1999 and previously situated in the University of Medicine and Dentistry of New Jersey (UMDNJ), will continue its mission of establishing a Center of Excellence in the State where basic science and clinical research studies, and clinical diagnosis and treatment initiatives can take place. To this end, the Council will award grants and contracts to public and private nonprofit entities.

In 2008, the Council funded the second year of its $3.3 million Basic Science and Clinical Research grant initiative, and launched a 2-year, $6 million dollar Clinical Enhancement grant program designed to improve families’ access to services, reduce wait times for those receiving developmental evaluations, and increase the number of children that can be assessed by multidisciplinary evaluation teams.

As of December 31, 2008, $3 million dollars have been budgeted and processed for the six Clinical Enhancement Center grants. From July 1 to December 31 of Fiscal Year 2009 the grantees expended $1,406,804 for Autism services, and the remaining $1,609,016 in funding will be allocated to the six centers in monthly installments over the course of the fiscal year.

Council Membership and Staff

Dr. Michael Gallo, Jr. was appointed the Executive Director of the Governor's Council in May, 2008. Dr. Gallo also served in this capacity when the Council was situated at UMDNJ.

P.L. 2007, Chapter 168 expanded the Council’s membership from a six-member to a fourteen-member board. The Council’s membership consists of representatives from academic institutions, autism and healthcare organizations, appointees of the Senate President, Assembly Speaker and Commissioner of Health, and also includes a member from the general public, and an individual with autism, or family member. Four positions remain vacant on the Council, as the Governor’s office still needs to name and vet 2
individuals to become Academic Institution representatives, 1 to become the fourth and final Autism Organization representative, and 1 to be the Public Member representative.

The first meeting of the reconstituted Governor’s Council for Medical Research and Treatment of Autism was held on December 8, 2008 at the Mercer County Library. At the meeting, Dr. Caroline Eggerding, the Executive Vice President of Pediatric and Adolescent Services for Bancroft Neurohealth, and the State Assembly Speaker Appointee to the Council, was elected Chair of the Council. At this meeting, the Council also voted to earmark $5 million for a new round of grants in the Basic Science and Clinical Research grant program. The expectation is that the RFA for this grant initiative will be announced in April, 2009.

Please see Appendix A for a list of the current Council members and vacancies on the Council, the Council staff, the 2009 Council meeting schedule, and the ratified Rules of Order governing the operation and functioning of the Governor’s Council.

Grant Initiatives

Basic Science and Clinical Research grants
The Council is currently funding the second year of its $3.3 million, Basic Science and Clinical Research grant program. This initiative, established in January 2007 when the Council was housed at the UMDNJ, funds 11 grants in the areas of genetic-environmental interactions, oxidative stress, epidemiology and population science.

Please see Appendix B for a listing of the 11 Council-funded research grants, the abstracts and first-year status reports for these projects, and the peer-reviewed and papers to be submitted for publication that were generated by the 2007 Council funding.

In April 2009 the Council will be announcing an RFA for a new cycle of Basic Science and Clinical research grants. This $5 million grant initiative will fund up to ten grants at a funding level of up to $500,000 over two years.

Clinical Enhancement Center grants
The Council is currently funding the first year of its 2-year, $6 million dollar Clinical Enhancement Center grant initiative. This program enables clinical autism centers across the State of New Jersey (there are 2 centers each in Northern, Central and Southern New Jersey), to enhance their staffing of clinical personnel, and to increase number of multi-disciplinary evaluations that they can provide to children suspected of being on the autism spectrum. The goals of this project are to increase families’ access to clinical services, to collect individual-level data on the patients being seen at the centers, and to coordinate the centers to create a foundation by which to attract federal funding for a State of New Jersey Autism Center of Excellence.
The 6 centers that are funded through the Clinical Enhancement Center grant initiative are:

- Children’s Specialized Hospital in Toms River
- Jersey Shore University Medical Center in Neptune
- The Autism Center of New Jersey Medical School at UMDNJ in Newark
- The Center for Neurological and Neurodevelopmental Health II (CNNH) in Voorhees
- The Child Development Center at Hunterdon Medical Center in Flemington
- The Institute for Child Development at Hackensack University Medical Center

The executive Director of the Governor’s Council convened a meeting of the Principal Investigators of the six Clinical Enhancement Center grants on June 24, 2008. The meeting was held at the Children’s Specialized Hospital in New Brunswick, and the meeting provided Division of Family Health Services personnel and the grantees to discuss the Centers’ enhancement plans and the need to collect patient-level data through a collective database.

Please see Appendix C for a list of the Clinical Enhancement Centers and the Power Point slides of each of their enhancement plans.

An important aspect of the Clinical Enhancement Center grant program is that it provides the opportunity to collect patient-level across six clinical sites. This data will be entered by the individual Centers into an Access database that was designed by Software Development Specialist Assistant, Mr. Anthony Mosco of the Division of Family Health Services (Please see Appendix D for the information page on how to use the Birth Defects Registry/Governor’s Council on Autism Registration Software, and web shots of the fields included in the database). This database is significant in that it is the first statewide autism database in the country, and it will be an extremely powerful tool because it will provide basic and clinical researchers in the State with sampling populations, and with demographic data on the Autism Spectrum Disorder (ASD), patients in the State of New Jersey, data on expressed phenotypes, and information on the process by which clinicians arrive at a diagnosis. In late January, 2009 Research Scientists, Drs. Sandra Howell and Nancy Scotto-Rosato will begin visiting the Clinical Enhancement Centers to install the Access database and to discuss with the Centers’ Data Coordinators the different reporting requirements for the Birth Defects Registry and the Governor’s Council’s Collective database information.

Governor’s Council Website

The website for the Governor’s Council for Medical Research and Treatment of Autism will be published on the Division of Family Health Services website in mid January, 2009. Dr. Gallo, with input and advisement from Senior Public Health Physician, Dr. Marilyn Gorney-Daley, and Research Scientist 1, Mr. Chuck Denk, designed the content
of the website, and the URL for the site is, “NJ.gov/health/autism.” The site was designed to address the needs of the various stakeholders in the New Jersey autism community.

The website contains the following elements:

- An overview of the purpose and mission of the Council
- Frequently asked questions about autism
- A listing of the Council members and Council staff
- The Autism Council’s policies and bylaws
- The meeting schedule for the Council
- The meeting agenda for the December 8, 2008 Council meeting
- The December 8, 2008 Council meeting minutes
- Information on research funding opportunities
- Listings of the current initiatives supported by the Council
- An autism resources/links page
- Contact information for the Executive Director of the Council

Please see Appendix E for the web shot of the front page of the Governor’s Council’s website.

Future Directions

Grant funding activities
The Council has submitted Fiscal Year 2010 Notice of Grant Awards for $3 million for the second year of its Clinical Enhancement Center program, $2.5 million for the first year of its new cycle of Basic Science and Clinical Research grants, and $500,000 for a postdoctoral fellowship program. It is anticipated that the Council will vote to authorize the Request for Applications (RFA) for the Basic Science and Clinical Research grant program at its April, 2009 meeting. In the early 2009, Dr. Gallo will be recruiting a 5-member Scientific Advisory Board to assist the Council in determining the type of grant programs to sponsor, and to review Council-sponsored research. Dr. Gallo will be recruiting an individual to Chair the Basic Science and Clinical Research grant review process, and will also be working with that individual to recruit reviewers to assess the quality of the grant applications that the Council receives. It is the council’s expectations that funding can be forwarded to the Principal Investigators who are awarded grants by the end of October, 2009.

Collective Database Development
The Council will be collecting patient-level data from its Clinical Enhancement Centers. This data collection process will enable the Council to report on the number of patients seen by these centers, across variables such as patient age, gender and diagnosis. The database that will contain this data will enable the Council to demonstrate the impact that its grant funding is having on the clinical autism activities in the State. The data
collection process will greatly aid researchers in being able to locate specific sample populations for research projects and clinical initiatives.

**Outreach and Grant Monitoring Activities**

In June 2009 The Council will host the second annual meeting of the Principal Investigators of the Clinical Enhancement Centers. This meeting will enable the Investigators to share information on best practices and lessons learned. In addition, the Council will be developing a listserv to allow the Centers to participate in threaded discussions. The Council also plans on hosting a scientific research conference in May 2010. This conference will focus on the Council-funded basic science and clinical research projects and treatment initiatives funded by the Council over the past 5 years. In order to effectively plan this conference, the Council will contract with a conference planner to manage the logistical aspects of this event.
APPENDIX A

- List of current Council members and vacancies
- The Council staff
- The 2009 Council meeting schedule
- The ratified Rules of Order of the Governor’s Council for Medical Research and Treatment of Autism
LIST OF COUNCIL MEMBERS AND VACANCIES
The Governor’s Council for
Medical Research and Treatment of Autism

Current Composition of the Council

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

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

**Ms. Caroline Eggerding, M.D.**, Assembly Speaker Appointee: Executive Vice President of Pediatric and Adolescent Services for Bancroft NeuroHealth.

**Linda S. Meyer, MPA, Ed.D.**, Autism Organization Representative 1; Executive Director of the NJ Center for Outreach and Services for the Autism Community (COSAC).

**B. Madeleine Goldfarb**, Autism Organization Representative 2; Founder/Director of the Noah’s Ark Children’s Association.

**Jessica C. Guberma, Ph.D.**, Autism Organization Representative 3; Executive Director for Community Options, Inc.

**VACANT**

Autism Organization Representative 4

**Barbie Zimmerman-Bier, M.D.**, Academic Institution Recommendation 1; University of Medicine and Dentistry – New Jersey Medical School

**Kendell R. Sprott, M.D., J.D.**, Academic Institution Representative; University of Medicine and Dentistry – New Jersey Medical School

**VACANT**

Academic Institution Recommendation 3

**VACANT**

Academic Institution Recommendation 4

**Yvette Janvier, M.D., FAAP**, Health Care Organization Representative; Medical Director for the Children’s Specialized Hospital in Toms River.

**VACANT**

Public Member

**Grace M. Reilly, RN, MSN, APN-C**, Individual with Autism or Family Member; Adult Nurse Practitioner for Riverview Medical Center.
THE COUNCIL STAFF
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Division of Family Health Services

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THE 2009 COUNCIL MEETING SCHEDULE
The Governor's Council for Medical Research and Treatment of Autism

Council Meeting Calendar

<table>
<thead>
<tr>
<th>Meeting Date</th>
<th>Location</th>
<th>Time</th>
<th>Agenda or Minutes</th>
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| December 8, 2008   | Mercer County Library  
                      2751 Brunswick Pike  
                      Lawrenceville, NJ 08648 | 6pm – 9pm | Agenda           |
| February 2, 2009   | NJ Department of Human Services, Division of Developmental Disabilities  
                      Conference Room 199A  
                      5 Commerce Way, Hamilton 08691 | 6pm-8pm | Agenda           |
| April 6, 2009      | NJ Department of Human Services, Division of Developmental Disabilities  
                      Conference Room 199A  
                      5 Commerce Way, Hamilton 08691 | 6pm-8pm | Minutes          |
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<td>June 1, 2009</td>
<td>NJ Department of Human Services, Division of Developmental Disabilities</td>
<td>6pm-8pm</td>
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<td>5 Commerce Way, Hamilton 08691</td>
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<td>August 3, 2009</td>
<td>NJ Department of Human Services, Division of Developmental Disabilities</td>
<td>6pm-8pm</td>
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<td>Conference Room 199A</td>
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<td>5 Commerce Way, Hamilton 08691</td>
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<tr>
<td>October 5, 2009</td>
<td>NJ Department of Human Services, Division of Developmental Disabilities</td>
<td>6pm-8pm</td>
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<td>Conference Room 199A</td>
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<td>December 7, 2009</td>
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THE RATIFIED RULES OF ORDER OF THE GOVERNOR'S COUNCIL
FOR MEDICAL RESEARCH AND TREATMENT OF AUTISM
GOVERNOR’S COUNCIL FOR MEDICAL RESEARCH
AND TREATMENT OF AUTISM

Rules of Order

January 2009
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INTRODUCTION

On September 12, 2007, New Jersey Governor, Jon Corzine, signed P.L. 2007, Chapter 168 into law establishing the Governor's Council for Medical Research and Treatment of Autism in the New Jersey Department of Health and Senior Services (DHSS). In moving to the DHSS the Council, which was established by State Statute in 1999 and previously situated in the University of Medicine and Dentistry of New Jersey (UMDNJ), is to continue its mission of establishing a Center of Excellence in the State where basic science and clinical research studies, and clinical diagnosis and treatment initiatives can take place. To this end, the Council will award grants and contracts to public and private nonprofit entities. P.L. 2007, Chapter 168 also expanded the Council's membership from being a six-member to a fourteen-member board. The Council's membership is made up of representatives from academic institutions, autism and healthcare organizations, appointees of the Senate President, Assembly Speaker and Commissioner of Health, and also includes a member from the general public, and an individual with autism, or family member.

ARTICLE I: MISSION AND PURPOSE

Section 1. Name

The Council is established in P.L.2007, Chapter 168 as the Governor's Council for Medical Research and Treatment of Autism (hereinafter referred to as “Council”).

Section 2. Authority and Primary Purposes


The Council was created to establish a Center of Excellence in Autism in the State of New Jersey.

As stipulated in P.L.2007, Chapter 168, the Center shall use the facilities of a single medical facility or higher education medical institution, or be formed from a consortium of cooperating facilities or institutions, and shall meet any requirements as may be prescribed by the council, with the understanding that the work carried out at the center shall be comprehensive and fully collaborative.

The mission of the Council is to make awards of grants and contracts to public and nonprofit private entities where basic and applied biomedical research, diagnosis and treatment for autism shall take place. It is the purpose of the Council to define the scope of the grant programs undertaken by the Center.
Governor’s Council for Medical Research

Section 3. Goals of the Council (as stipulated in P.L. 2007, Chapter 168)

- To establish a Center of Excellence in the State of New Jersey.

- To forward the understanding of the potential causes and treatments of Autism Spectrum Disorders (ASDs) by supporting high quality basic science studies and clinical research initiatives.

- To identify strategies for studying autism and developing areas of research.

- To facilitate the enhancement and development of clinical and educational centers to offer individuals with autism, and their families, greater access to diagnostic and treatment services by developing and monitoring grant initiatives.

- To develop an autism database that will catalogue the diagnostic data of individuals with autism throughout the State.

- To disseminate the status reports for, and findings of Council-funded studies on the Council’s website so that they are accessible to autism organizations, the New Jersey autism community, healthcare professionals who treat individuals with ASDs, and to those responsible for legislative and policy development.

- To provide support to autism organizations to assist them in sponsoring conferences, seminars and workshops which are in line with the goals of the Council.

- To identify additional funding sources for the Council.

- Additional goals will be set as determined by the Council

ARTICLE II: COUNCIL RESPONSIBILITIES AND DUTIES

Section 1. Responsibilities and Duties

- Provide guidance to the Director of the Council regarding the Director’s goals, in order to ensure consistency with the Council’s mission, goals and objectives.

- Vote to approve candidates for the Council’s five-member Scientific Advisory Board

- Vote on the recommendations made by the Council’s Scientific Advisory Board regarding which grant applicants should receive Council funding.

- Approve Council meeting minutes and agendas.
Governor's Council for Medical Research

- Develop a comprehensive website that provides pertinent information to all of the stakeholders in the New Jersey autism community.

- Review and monitor the progress of Council-funded grant projects.

- Assess the need for demonstration projects and provide management for approved projects, when appropriate.

- Conduct such other activities as the Council deems appropriate and that are in accordance with the enabling legislation, in order to advance the importance of research into the causes of, and treatments for, autism spectrum disorders, and to provide information to the public and scientific communities about the Council's activities as they regard the advancement of autism research.

ARTICLE III: COUNCIL MEMBERSHIP AND STRUCTURE

Section 1. Composition of the Council

The Governor's Council on Medical Research and Treatment of Autism is composed of fourteen (14) members, to be appointed in accordance with the enabling legislation.

Section 2. Conduct of Council Members

The Council is comprised of a diverse group of family members, as well as a variety of health and human service, autism organization, policy and education representatives. As a collective body, the membership addresses procedural, programmatic, legislative or administrative issues affecting people with autism spectrum disorders. To facilitate the full Council's efforts, the Council asks individual members to meet the following expectations:

A. All Members:

1. For the Council to perform its functions, each member needs to attend and actively participate in the Council's activities. Members should also provide ongoing input and guidance on the efforts of the Council. The Council wants all of its members to be involved in developing and implementing policies.

2. To effectively address issues affecting people with autism spectrum disorders, the Council must rely on the expertise and experience of its individual members. The Council wants its members to offer technical assistance and personal insights on matters about which they possess expertise or experience.

3. To be effective, the Council needs outside persons and organizations to be aware of its activities and provide input. Accordingly, the Council wants its members to disseminate information about Council activities to persons and organizations that would benefit from
knowledge about the Council or be able to facilitate the Council’s goals. However, individual Council members also need to ensure that information is accurately and fairly communicated to others. A Council member should be cautious in representing the Council’s positions and should only represent as the Council’s positions those positions that have been agreed to by the Council. Only the Chair and/or the Director (at the direction of the Chair), should give press interviews or issue public statements representing the Council.

B. Members Who Represent Agencies or Academic Institutions:

1. For the Council to meet its legislative mandates, the Council needs to know about the policies and activities of the agencies and academic institutions represented on the Council. Accordingly, the Council needs its members who represent agencies to inform the Council about their agencies’ policies and activities as they related to people with autism and other pervasive developmental disorders.

2. If a member who represents an agency or academic institution cannot attend a Council meeting, the Council wants that member to send an alternate, non-voting representative of the agency, if possible. This representative will not participate in the Council’s deliberations, but will be present to ensure that the absent member, and the appropriate individuals from that member’s academic institution/agency, are kept fully informed regarding the Council’s activities.

3. Members who represent agencies or academic institutions need to act as liaisons between the Council and their agencies. These members should communicate Council activities to their agencies’ administrative, operational, and governing personnel and communicate relevant activities of their agencies to the Council. This type of communication should be routine to allow the other Council members to provide input regarding the activities of the agencies and the agencies to provide input regarding the activities of the Council.

4. Although the Academic Institution members of the Council have unique interests in the area of ASDs, it is important for these members to remain cognizant of the needs and interests of all citizens of New Jersey and ensure that the Council’s policies are sound public policies for all.

C. Public Members Appointed by the Governor, Senate or General Assembly:

1. Although the public members of the Council do not officially represent any constituency, they are the public face of the Council. Accordingly, the Council wants its public members to collect information from the public regarding the Council’s activities and report it to the Council.

2. Although the public members of the Council have unique interests in the area of ASDs, it is important for these members to remain cognizant of the needs and interests of all
Governor’s Council for Medical Research

citizens of New Jersey and ensure that the Council’s policies are sound public policies for all.

Section 3. Absences of Council Members

A. The Council requests that members notify the Director and/or the Chair of any necessary absence from a meeting at the earliest possible opportunity before the meeting.

Section 4. Grounds for Recommending Removal

It is grounds for recommending the removal of a member from the Council if that member:

1. exhibits behavior that is illegal or unethical as a member of the Council;

2. cannot, because of illness or disability, discharge the member’s duties for a substantial part of the member’s term

3. is absent from more than half of the Council meetings that the member is eligible to attend during a calendar year without providing notification to the Council prior to such absences;

4. consistently fails to meet the expectations contained in these policies and procedures.

ARTICLE IV: OFFICERS OF THE COUNCIL

Section 1. Chair

At its first meeting of each calendar year, the Council shall select, by a simple majority of the members present, a chairperson from among its members, who shall serve as the chairperson until the first meeting held in the next calendar year, at which time the same person may be selected as chairperson or a new chairperson may be selected in the same manner.

Section 2. Vacancies in Position of Chair

In the event of a vacancy for the position of Chair, a meeting of the Council will be arranged to have the council members select, by a simple majority of the members present, a new chairperson from among its members.

Section 3. Duties and Responsibilities of the Chair

The Chair shall preside at all meetings of the Council. The Chair shall appoint the Chairs of any committees. The Chair shall exercise general supervision over the work of the other Council members and Director to assure that the mission, goals, and policies of the
Governor’s Council for Medical Research

Council are adhered to. The Chair is responsible for ensuring that all duties and directives set forth in the Council’s authorizing legislation are adhered to by the Council. The Chair will be responsible for directing the creation of the Annual Council report, and ensuring that the report is submitted to the Governor’s Office and the legislature detailing the Council’s activities regarding its legislative mandate and administrative directives. The Chair shall be the official representative of the Council and shall present testimony, conduct negotiations, approve changes or submissions to the web site, represent the Council at public meetings and conferences, and participate in formal deliberations on behalf of the Council. The Chair may officially represent the Council and may carry out actions authorized by the Council in a properly convened open meeting.

Section 4. Chairs of subcommittees

Committee and Task Force Chairs are responsible for ensuring that their committee responds to all issues that fall under the oversight of that specific entity. Chairs will be responsible for providing regular reports to the Council.

ARTICLE V: COMMITTEES AND ADVISORY TASK FORCE

Section 1. Council Committees

The Council may establish standing, special, ad hoc, and interim committees of Council members to expedite the work of the Council. Council members and Chairs of such committees shall be appointed by the Chair.

A. Council Committee Criteria:

1. The Chair shall appoint the Chairs of any committees.

2. A committee shall transact business in any manner calculated to expedite its work.

3. The Chairs of each committee are responsible for presenting any findings or recommendations made by their members to the full Council.

4. Written notice of each committee meeting shall be provided to all committee members at least 10 days prior to each meeting. Such notice shall include the time, date, place, and items to be discussed at that meeting.

5. The Chair may appoint an Ad-hoc Committee, which shall be appointed on a temporary basis by the Chair to address a specific issue or problem that includes a clear charge of duty and an established date of completion. Ad-hoc Committees will keep the Chair and Council appraised of their work and submit final reports at the conclusion of their work to the Council.
ARTICLE VI: CONDUCT OF COUNCIL MEETINGS

Section 1. Required Meetings

The Governor’s Council for Medical Research and Treatment of Autism shall meet at least four times a calendar year. All meetings of the Full Council shall be conducted according to Robert’s Rules of Order, Revised, except as provided in these policies and procedures.

Section 2. Additional Meetings

The Council may meet at other times at the call of the Chair or as provided by Council motion or rule.

Section 3. Quorum

A quorum of the Council consists of a majority of appointed members of the Council. A quorum for committee and Task Force meetings shall be a simple majority of the active members of the committee or Task Force.

Section 4. Applicability of Open Public Meetings Law

A. The Council in all meetings is subject to the requirements of the Open Public Meetings Law (N.J.S.A. 10:4-6). The Council’s records are subject to the Open Public Records Act. The official minutes of all Council, Task Force, and committee meetings are kept in the office of the Director which offers administrative support for the Council, and are available for public review as authorized by the Open Public Meetings Act.

B. A committee or Task Force may meet by teleconference call providing that the entity is only making recommendations to the full Council.

Section 5. Meeting Agenda

A. The Chair, in collaboration with the Director, shall prepare the agenda for each Council meeting. The Chair should give due consideration to all Council members and the Director for placement of items on the Council’s agenda.

B. The Open Meetings Law requires a subject to be placed on the Council’s agenda before the Council consider that subject. Any individual or organization not represented on the Council who desires to have a subject placed on the agenda of a Council meeting shall make a request to do so by contacting the Director of the Council.

D. The request to have a subject placed on the agenda of a Council meeting shall:

1. state the subject to be placed on the agenda;
2. state the time required to provide presentation at the full Council meeting; and

3. be made at least fourteen days before the date of the Council meeting at which the requestor wants the subject discussed. Any request made the agenda deadline will be considered for the agenda of the next Council meeting. If the Chair of the Council deems the topic appropriate for discussion by the Council, the request to have a subject placed on the meeting agenda will be granted.

E. The final agenda, and all supporting materials shall be disseminated by the Director, either electronically or via U.S. Mail, to all members of the Council a minimum of seven (7) calendar days prior to the council meeting at which the items on the agenda are to be considered.

Section 6. Notice of Meetings

A. Written notice of time and place of each Council meeting shall be made to the public.

B. In accordance with the Open Public Meetings Act, notices to at least two (2) New Jersey newspapers (the Council will use the Newark Star Ledger, The Trenton Times and the Courier Post), for posting shall be submitted seven (7) full 24-hour periods prior to an impending meeting excluding the day of posting and the day of the meeting.

C. A copy of the notice of each Council meeting shall be sent to each member at least seven (7) days prior to the meeting except in emergency cases; as determined by the Chair.

Section 7. Transaction of Business

A. All meetings of the Full Council shall be conducted according to Robert’s Rules of Order, Revised, except as provided in these policies and procedures.

B. All Council actions taken must be approved by a simple majority vote of the members present.

Section 8. Public Access

Opportunities to provide public comments are provided at each Council meeting, and such public comment periods are to be listed on the agenda for each Council meeting. The Chair of the Council may limit each person presenting public comments or public testimony on any agenda item to a certain number of minutes by announcing the period when comments or testimony are given.
Section 9. Minutes

The Director shall be responsible for the creation and maintenance of the records and minutes of the Council. The Director shall assure that approved minutes are distributed to the members of the Council and to such others as the Council may direct.

Section 10. Amendment of Policies

Council policies may be adopted or revised by a majority of the members present at a regular or called Council meeting providing a quorum is present at that time. Written notice of the proposed amendment(s) must be provided to Council members at least ten (10) days prior to any such action. Amendments may be proposed by a recommendation of the Chair or by written request of any three (3) members of the Council.
APPENDIX B

- The eleven Council-funded Basic Science and Clinical Research grants
- The abstracts of the Council-funded Basic Science and Clinical Research grants
- The first-year status reports for the Basic Science and Clinical Research grants
- Peer-reviewed and papers generated by the 2007 Council-funded Basic Science and Clinical Research grants
- Papers to be submitted for publications that were generated by the 2007 Council funding
THE 2007 BASIC SCIENCE AND CLINICAL RESEARCH GRANTS
Governor's Council for Medical Research and Treatment of Autism

2007 Basic Science and Clinical Research Proposals

The Basic Science and Clinical Research program is designed to sponsor research into the cause, diagnosis, early detection, prevention, control and treatment of autism. This grant program supports research in a variety of fields, including (but not restricted to), genetics, neurobiology, epidemiology, neuroimaging, psychopharmacology, immunology, environmental health, infectious diseases, gastroenterology, and endocrinology. The 11 basic science and clinical research grant projects that were funded by the Council in January 2007 are:

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Title</th>
<th>Institution</th>
<th>Type of Study</th>
<th>Funding Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Emmanuel DiCicco-Bloom</td>
<td>Functional Characterization of the Autism-Associated Gene, Engrailed-2</td>
<td>RWMS/Neuroscience and Cell Biology</td>
<td>Basic Science</td>
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<td>2) Michael Lewis</td>
<td>Brain Maturation and Self Representation in Young Children with Autism Spectrum Disorder</td>
<td>RWJMS/Institute for Child Development</td>
<td>Clinical</td>
<td>$295,821</td>
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<tr>
<td>3) John Pintar</td>
<td>Opioid System Contributions to Autism Linked Behavior</td>
<td>RWJMS/Neuroscience</td>
<td>Basic science</td>
<td>$299,930</td>
</tr>
<tr>
<td>4) Gleb Shumyatsky</td>
<td>Amygdala-Enriched Genes may be Involved in Gating Behaviors that are Critical for Survival</td>
<td>Rutgers/Genetics</td>
<td>Basic Science</td>
<td>$300,000</td>
</tr>
<tr>
<td>5) T. Peter Stein</td>
<td>Oxidative Stress and Brain Metabolism in Autism</td>
<td>UMDNJ-SOM/Surgery</td>
<td>Basic science</td>
<td>$299,478</td>
</tr>
<tr>
<td>6) Pauline Thomas</td>
<td>Understanding the scope of autism in New Jersey Characteristic of children diagnosed with autism Spectrum disorder by the age 8 years, NJ</td>
<td>NJMS/Obgyn</td>
<td>Epidemiology</td>
<td>$202,014</td>
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<td>7) George Wagner</td>
<td>Protection against early toxicant exposure in a mouse model of autism</td>
<td>Rutgers/Psychology</td>
<td>Basic Science</td>
<td>$224,324</td>
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<td>Institution</td>
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<tr>
<td>8) Daniel Wartenberg</td>
<td>An Exploratory Epidemiologic Study of Autism in New Jersey and Some Possible Environmental Risk Factors</td>
<td>UMDNJ/EOHSI</td>
<td>Epidemiology</td>
<td>$299,982</td>
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<tr>
<td>9) Walter Zahorodny</td>
<td>New Jersey Autism Study: Population-Based Surveillance of Autism Spectrum Disorder in New Jersey</td>
<td>NJMS/Pediatrics</td>
<td>Epidemiology</td>
<td>$300,000</td>
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<tr>
<td>10) Walter Zahorodny</td>
<td>Young Adults with Autism: A Pilot Epidemiologic Investigation</td>
<td>NJMS/Pediatrics</td>
<td>Epidemiology</td>
<td>$203,628</td>
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<td>11) Steven S. Zalcman</td>
<td>Treatments for the prevention of autistic-like Behavior in offspring of mothers infected With influenza virus during pregnancy: An animal model</td>
<td>NJMS/Psychiatry</td>
<td>Basic Science/Translational</td>
<td>$299,765</td>
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THE ABSTRACTS OF THE COUNCIL-FUNDED BASIC SCIENCE AND CLINICAL RESEARCH GRANTS
Project Title: Functional Characterization of the Autism-Associated Gene, *Engrailed-2*

**ABSTRACT:**

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disease characterized by deficits in language, sociability, and behavior. The etiology of ASD is largely unknown, however strong evidence suggests high heritability, with approximately 3-15 susceptibility genes responsible for the majority of ASD. The heterogeneity of symptoms suggests ASD may be a collection of endophenotypes, in which distinct genetic abnormalities segregate with specific deficits. However, postmortem and structural imaging studies consistently report cerebellar abnormalities, due to dysregulated growth and reductions of Purkinje and granule neurons. Previously, we reported association of the cerebellar patterning gene, *ENGRAILED-2 (EN2)*, with ASD in three separate populations (p ≤ 0.000005), contributing 40% risk within ASD populations. Interestingly, *En2* knockout (KO) mice show reduced sociability, cerebellar vermis hypoplasia and neuron deficits, similar to human ASD. Thus, *En2* KO mice represent a relevant genetic animal model for studying ASD, cerebellar development, and the currently undefined role of *En2* in both. To characterize *En2* in granule neuron precursor (GNP) proliferation, differentiation and survival, we propose to: 1) Compare *En2* KO and wildtype (WT) mouse GNP DNA synthesis and differentiation in vitro without and with developmentally relevant growth factors, 2) Define changes in differentiation following *En2* over expression and RNAi knockdown in GNPs, 3) Examine genotype-dependent differences in GNP proliferation in vivo, and effects of administering to newborns growth factors defined in culture to be differentially regulated by *En2*. Our preliminary data suggest *En2* facilitates cell cycle exit and promotes differentiation, both in cultures and living animals.

**Principal Investigator:**

Emanuel DiCicco-Bloom, MD
Project Title: Brain Maturation and Self Representation in Young Children with Autism Spectrum Disorder

ABSTRACT:

A striking clinical feature of children with Autism Spectrum Disorders (ASD) is social-interpersonal communication failure characterized by poor relationships between the self and others. In addition, research has revealed brain abnormalities in children with ASD. The aim of this project is to study the relation between brain maturation and social, emotional, and cognitive development in children with ASD. Our pilot data show the relation between brain maturation and social development in children. We have evidence that the emergence of self representation is related to brain maturation in typically developing children, and to deficits in children with ASD. Based upon our past research, we predict that the amount of self representational behavior in ASD children will be related to brain maturation, specifically to the maturation of the superior temporal gyrus and the temporo-parietal junction. In order to measure brain maturation we will obtain clinical magnetic resonance images (MRI) and use our programs to quantify myelination development. We hypothesize that the degree of maturation will be related to the child’s development of self representation. This research will lead to a better understanding of the factors contributing to failures of self representation in children with ASD.

We plan to measure these relations in children at the Family Health Center of the Jersey Shore University Medical Center in Neptune, New Jersey. At the time of their visit to the MRI facility, parents will be recruited. We will use the clinical MRI images to measure brain maturation, and within one month of the MRI examination, we will assess self representational behavior.

Principal Investigator:

Michael Lewis, Ph.D.
Project Title: Opioid System Contributions to Autism Linked Behavior

ABSTRACT:

Endogenous peptide systems and the underlying reward circuitry have recently gained attention as targets that, when altered, can lead to autistic-like behaviors in mice. We propose to test the effects of several opioid system ligand and receptor mutations we have produced on mouse social behaviors currently thought to reflect distinct aspects of human autism spectrum disorder (ASD). These experiments will allow us to begin to test the hypothesis that multiple opioid system components contribute to the ASD-like phenotype. We will first determine whether mu (MOR-1) opioid receptor KO mouse pups exhibit gene-dosage and/or background dependent alterations in attachment and/or social behavior using both pup ultrasound vocalization and a neonatal social learning assay in which sensory and somato-sensory stimuli are coupled. If alterations are observed, we will then identify the anatomic sites where neonatal maternal deprivation leads to MOR-1 activation. We will complement these studies of the neonate by using a newly-developed three chamber choice test to assess social motivation and preference behavior in the adult MOR-1 KO and complement this behavioral analysis by extending preliminary studies that indicate an increase in astrocyte number in adult MOR-1 KO. As time permits, we will begin to determine whether KOs of the endogenous MOR-1 ligands enkephalin and /or endorphin mimic any MOR-1 KO deficits in attachment or other behaviors and, finally, begin to extend behavioral study of the endogenous opioid system to the delta and kappa opioid receptor systems, which we have shown contribute differentially to the reward pathway. Together, these studies will begin to define the extent of opioid system involvement in development of behaviors currently thought to reflect those altered in ASD and thus produce preliminary data to support future R-21 or R-01 applications to NIMH.

Principal Investigator:

John Pintar, Ph.D.
Project Title: Amygdala-Enriched Genes May Be Involved in Gating Behaviors that Are Critical for Survival

ABSTRACT:

Our new proposal represents an extension of our research program: in addition to studying fear, we will be studying how fear affects other behaviors that may or may not be directly dependent on the amygdala but nevertheless are affected by anxiety or fear. We have recently identified and characterized two amygdala-enriched genes that control amygdala function in opposite directions. In this proposal using the knockouts of these genes in mice, we will study how amygdala deficiency or enhancement modulates other behaviors that are affected in the presence of danger, such as panic-like responses, maternal behaviors and social behaviors. To this end, we will examine how these behaviors are modulated by 1) elimination of some of the principal neurons in the amygdala, 2) gene knockout that increases amygdala synaptic plasticity and fear memory and 3) gene knockout that decreases amygdala synaptic plasticity and fear memory. These behaviors will be analyzed in the presence of highly emotional stimuli, which represent a potential threat to an organism. We will also test whether the elimination of amygdala-enriched genes leads to changes in brain activity in the amygdala and brain areas connected to it. We will use c-fos imaging to analyze brain activity in naïve state and after electric footshock in Stathmin, GRP and GRPR knockout mice. In summary, our proposed experiments will test the hypothesis that amygdala-enriched genes are involved in establishing a threshold by which the amygdala evaluates the level of danger and thus gates behavioral responses. While this work is analyzing anxiety-dependent behavioral responses in mice with the deletions of specific amygdala-enriched genes, the results may have implications for disorders that have anxiety as their component, such as autism, phobias and panic disorders.

Principal Investigator:

Gleb P. Shumyatsky, Ph.D.
Project Title: **OXIDATIVE STRESS AND BRAIN METABOLISM IN AUTISM**

**ABSTRACT:**

We, and subsequently others have shown that oxidative stress as measured by isoprostanate excretion is increased in children with autism. Isoprostanates are derived from the auto-oxidation of arachidonic acid (AA). We propose that this is part of a systemic increase in auto-oxidation of PUFAs. DHA is a major brain lipid. As such the products of DHA auto-oxidation have the potential of affecting processes within the brain. The aims of this proposal are: (i) To develop assays for detecting the products of DHA oxidation in urine. The specific DHA metabolites to be investigated are: iPF₁₀⁻VI, the resolvins D₂, D₄, D₅ and D₆ and neuroprotectin. We will synthesize these compounds with and without deuterium labeling and use them to develop a series of isotope dilution-gas chromatograph-mass spectrometric assays. (ii) To demonstrate that their excretion is altered with autism. This task will be done by collecting and analyzing urine from 40 freshly diagnosed cases of autism and 40 age matched controls.

**Principal Investigator:**

T.P. Stein, Ph.D.
Project Title: Understanding the scope of autism in New Jersey: Characteristics of children diagnosed with autism spectrum disorders by the age of 8 years, NJ, 2000-2002

ABSTRACT:

Background: During the past two decades estimates of autism prevalence have risen. This may represent a true increase or change in ascertainment and diagnosis. Risk factors for autism also remain unclear. A robust data set is now available, with the most complete possible ascertainment to date of children with autism spectrum disorder (ASD) in New Jersey (NJ). Resources are needed to analyze the data, to better understand the scope of autism in NJ children. Preliminary analyses suggest 1) 1 in 100 NJ children meet criteria for ASD, and 2) prevalence varies with socioeconomic status and county.


Methods: The dataset will be analyzed to test specific hypotheses about the prevalence and distribution of autism, including: 1) In NJ, autism prevalence does not differ by race/ethnicity; 2) ASD prevalence does not differ by socioeconomic level; and 3) Paternal age is associated with autism diagnosis in NJ. For this analysis, linked data from NJ Birth Certificates for case children will be compared with other children matched on age and county of birth.

Significance: These studies will enable completion of scientific work on data already collected. Full analysis of these data is essential to our understanding of the scope of autism in NJ, and for generating hypotheses on how best to intervene for optimal diagnosis and evaluation. Determining whether ASD is associated with paternal age will have implications for education and counseling.

Principal Investigator:

Pauline Thomas, MD
Project Title: Protection against early toxicant exposure in a mouse model of autism

ABSTRACT:

We have developed a novel animal model of autism which has proven to be sensitive, allowing us to categorize toxicant-induced neurobehavioral deficits along a developmental timeline and, independently, to detect effects of gene alteration on these same neurodevelopmental measures. In addition, we demonstrated that antioxidant pretreatment protects genetically-intact mice from toxicant-induced neurobehavioral deficits. Major limitations of this work, to date, have been the absence of morphological correlates of the toxicant-induced behavioral disruption and, of course, our failure to demonstrate that our antioxidant treatment is capable of protecting mice against such morphological damage induced by toxicants. Finally, we have yet to administer any of toxicants we have studied (metals, drugs, pesticides) to genetically-altered subjects. Accordingly, we now propose to administer our standard toxicant, valproic acid, to mice with altered expression of Engrailed2 (a gene involved in cerebellar development and highly associated with autism) and to assess these mice for deficits in neurobehavioral development as well as for morphological evidence of cerebellar cell damage. We then plan to prevent the occurrence of these toxicant-induced deficits by administration of Trolox, a water soluble derivative of vitamin E. We anticipate that En2−/− mice will be more sensitive to disruption of behavioral development and manifest more cerebellar cell damage than their wild-type controls and that Trolox administration will protect mice on both measures. These observations would have important ramifications for autism, known to be associated with alterations in Engrailed expression and cerebellar damage and thought to be triggered by early toxicant exposure.

Principal Investigator:

George C. Wagner, Ph.D.
Project Title: An Exploratory Epidemiologic Study of Autism in New Jersey and Some Possible Environmental Risk Factors

ABSTRACT:

Autism spectrum disorder (hereafter, ASD) is an extremely serious childhood condition. It is a highly variable, lifelong, multi-factorial, neurodevelopmental disorder that is characterized by impairments in social interaction, communication, and other behaviors. The prevalence of ASD has increased dramatically in the past few years nationwide, and in New Jersey, due in part to increased awareness, in part to better diagnoses, and possibly due to changing environmental conditions. Etiologically, ASD is believed to have strong genetic and nongenetic risk factors. Recent studies exploring possible environmental etiologies have reported provocative but unconfirmed associations. This study, using ASD prevalence data from an on-going CDC-funded surveillance project in New Jersey, will apply methods from an on-going CDC-funded Environmental Public Health Tracking project to describe the socio-demographic, spatial and temporal prevalence patterns of ASD in four counties in New Jersey. In addition, this study will assess the association of these prevalences with potential environmental risk factors, such as heavy metals and organic compounds, replicating and extending analyses conducted by other researchers. Further, we will assess the association of ASD with the concentrations of trihalomethanes in public drinking water (a result of the disinfection process), as has been suggested in a report by CDC’s Agency for Toxic Substances and Disease Registry (ATSDR). This project will clarify the patterns of ASD in New Jersey, be responsive to concerns regarding reported clustering, and provide insights into etiology.

Principal Investigator:

Daniel Wartenberg, Ph.D.
Project Title: New Jersey Autism Study: Population-Based Surveillance of Autism Spectrum Disorders in New Jersey

ABSTRACT:

Concern with high rates of autism calls for the accurate determination of baseline prevalence rates of autism and the ongoing monitoring of autism rates over time. A population-based, multiple source, public health surveillance investigation of Autism Spectrum Disorders (ASD) in a four county region of New Jersey, implemented by the investigators, has identified a very high prevalence of ASD. The autism prevalence rates identified by the New Jersey Autism Study for 2000 and 2002 were based on analysis of high quality data derived from multiple health and education records and constitute the most reliable New Jersey baseline autism prevalence estimates. Due to reduction in federal support of autism surveillance, autism monitoring in New Jersey has been discontinued, however.

The objectives of the proposed investigation are to establish the prevalence of autism for the cohort eight-year old children, born in 1998 and to describe the developmental and functional characteristics of these children for comparison with the (baseline) ASD rates and characteristics determined for cohorts of children born in 1992 and 1994.

Only by comparing the rates of autism and the expression of autism in different cohorts of children in a surveillance region, by the same methodology over time, can trends in the prevalence and expression of autism be accurately verified. Ongoing population-based surveillance of autism in New Jersey is essential for the scientific understanding of this important and complex developmental disability and for effective education, health and service planning on behalf of New Jersey’s children with autism.

Principal Investigator:
Walter Zahorodny, Ph.D.
Project Title: Young Adults with Autism: A Pilot Epidemiologic Investigation

ABSTRACT:

Recently, concern regarding an apparent increase in autism prevalence has intensified interest in understanding this disorder. To date, the vast majority of autism research has focused on the expression of autism in children. Only a single, community survey has attempted to identify the prevalence of autism among adults and to describe the needs of this group.

The objectives of the proposed investigation are to establish the prevalence of autism for a cohort of eighteen-year old adults in New Jersey and to describe the developmental and functional characteristics of these young adults for comparison with the prevalence and characteristics determined for different age cohorts.

Adults with Autism Spectrum Disorders (ASD), born in 1988, and residing in Union County, New Jersey will be ascertained through a valid, two-stage, epidemiologic method that includes screening and abstraction of health and education records at multiple sources and autism case determination by independent experts using DSM-IV-TR diagnostic criteria.

Obtaining accurate prevalence rates for autism in young adults will advance scientific understanding of this neglected group and fulfill an important public health need. By identifying the number of 18-year olds with autism in Union County and by describing the developmental and functional characteristics of these persons, information will be developed that can lead to improving the transition of adolescents with autism to adulthood and allow the informed development of special services for adults with autism.

Principal Investigator:
Walter Zahorodny, Ph.D.
Project Title: Treatments for prevention of autistic-like behavior in offspring

ABSTRACT:

Maternal influenza virus infection during the second month trimester increases the offspring’s vulnerability to psychiatric disorders, notably autism spectrum disorder (ASD) and schizophrenia. In parallel, behavioral disturbances and central nervous system (CNS) abnormalities reminiscent of those associated with ASD are evident in the offspring of mice exposed to influenza virus during the second trimester. Influenza virus-related neurobehavioral abnormalities are not due to the virus itself since it is not present in the brains of the infected mice. Instead, it is thought that elements of the maternal immune response, notably proinflammatory cytokines, are transformed to the fetus and act as precipitating agents. It is thus of unique interest that cytokines (notably proinflammatory cytokines and interferons) involved in the host’s anti-viral response are found in the fetus brains of infected mothers. Of further significance, these cytokines act as a potent neurodevelopmental factor by promoting neuronal survival, and neurite extension and growth in brain regions that develop during the second trimester and that are implicated in ASD. Thus, cytokine overexpression in the developing fetus during critical development period would have long-term repercussions on development of brain regions associated with ASD and on behavioral states that they subserve. Accordingly, we hypothesize that blunting the maternal cytokine response to influenza virus will prevent neurobehavioral disturbances in the offspring. We propose to treat infected pregnant dams with (a) monoclonal antibodies against proinflammatory cytokines or interferons; (b) reactive oxygen species, which blunt the anti-viral cytokines response and limit related pathology; or (c) vaccination against influenza virus.

Principal Investigator:

Steven S. Zalcman, Ph.D.
THE FIRST-YEAR STATUS REPORTS FOR THE BASIC SCIENCE
AND CLINICAL RESEARCH GRANTS
New Jersey Governor’s Council on Autism


Principal Investigator: Emanuel DiCicco-Bloom, M.D.

Hypothesis: We hypothesize that Engrailed-2 (En2) functions during postnatal cerebellar development to promote cell cycle exit and differentiation of granule neuron precursors, potentially acting through regulation of extracellular growth factor signaling.

We have made progress in each of the three Specific Aims of the grant, and provide titles of abstract presentations of the work.

Aim 1. Define the role of En2 in granule neuron precursor (GNP) proliferation, differentiation and survival by comparing En2 Knock Out (KO) and Wild Type (WT) mouse GNPs in culture, in the absence and presence of relevant developmental regulatory growth factors. Our studies now support a specific interaction between IGF1 and En2 genotype: In the absence of En2, GNPs respond to IGF1 mitogenic stimulation with a 2-fold greater increase in DNA synthesis than wild type (WT) cells in 24h cultures, as well as increased nuclear BrdU mitotic labeling. In marked contrast, other mitogens that engage tyrosine kinase receptors, such as FGF, EGF and BDNF, do not elicit genotype specific effects. Moreover, the major GNP mitogen, sonic hedgehog, stimulates DNA synthesis of both genotypes 2-3-fold, indicating that IGF1 signaling and En2 exhibit a specific interaction, which we are currently examining in detail at the level of second messenger signaling.

In addition to an enhanced proliferative response, we have begun to compare neuronal differentiation. While both WT and En2 KO GNP grow neurites in control conditions, the KO cells demonstrate a diminished neuritogenic response to IGF1. Specifically, IGF1 increases the percent of WT cells growing neuritic processes by 56%, whereas there is only a 23% increase in KO GNPs in response to the factor. In aggregate, the studies suggest that En2 promotes cell cycle exit and differentiation in association with IGF1, and in its absence, cells remain as mitotic precursors and exhibit less differentiation.

Aim 2. Evaluate the functional role of En2 in GNP cultures by altering gene expression levels using En2 cDNA transfection techniques and RNAi knockdown approaches. We have completed a series of experiments using En2 over-expression vector with results that are consistent with our proposal that the gene promotes cell cycle exit and neuronal differentiation. En2 over-expression at 24 hr results in reduction of several makers of proliferation including BrdU nuclear labeling, and expression of cell cycle protein, PCNA. In contrast, En2 over-expression resulted in twice as many cells elaborating long neuritic processes as well as expressing cytoskeletal protein, MAP1b, and transcription factor, Zic2, all indicating enhanced neuronal differentiation. Significantly, transfection effects were age-dependent: while En2 vector increased neurite outgrowth at both postnatal days (P) 4 and P7, effects were no longer observed at P10, when many more cells were already engaged in differentiation, suggesting that cell autonomous factors interact with En2 mechanisms.

Aim 3. Define the proliferative role of En2 in the developing mouse cerebellum, and characterize interactions with extracellular growth factor systems by peripheral or intracranial protein administration in the P7 mouse in vivo. We have now completed the assessment of BrdU mitotic labeling in the cerebellum of the En2 KO mouse. The labeling index (proportion of
BrdU+ cells/ total cells) in the KO was increased to 28.3% compared to the WT pups, where only 25.0% of EGL precursors were engaged in S phase. However, with further assessment, we found that the increase in labeling was entirely due to the cerebellar vermis, the central region, which exhibited a LI= 30.1% in the KO vs LI=23.2% in the WT, whereas the cerebellar hemispheres were no different. This region specific increase in mitotic labeling is consistent with the expression of En2 in cerebellum, which is restricted to the vermis alone at this age. These data suggest that as observed in vitro, En2 also promotes cell cycle exit in developing cerebellum, and in its absence more cells continue to divide.

Finally, we have begun to explore effects of IGF1 in vivo, and found that a single subcutaneous injection can stimulate DNA synthesis in whole cerebellum. Time course analysis indicates that increases are observed at 4, 8, and 12 hr, with the greatest effects at 8 hr, a time we have used as our paradigm. In the WT animal, IGF1 elicited a 12% increase in DNA synthesis. We are now in position to compare En2 KO to WT pups in the same experiment. We expect that IGF1 will stimulate a greater change in the KO, based on this kind of response in our culture models.

Our results indicate that En2 serves to promote cell cycle exit and neuronal differentiation both in vivo and in the culture model. In turn, abnormal regulation of EN2 during human development may result in changes in the numbers of cerebellar granule neurons produced, which may impact functions to contribute to autism spectrum disorders.

Over the next year we plan to continue the studies as originally proposed based on the successful progress reported above.
MICHAEL LEWIS, Ph.D.
University Distinguished Professor
Director

February 29, 2008

Michael Gallo, Jr., Ph.D.
Director
New Jersey Governor’s Council on Autism
UMDNJ-Child Health Institute
89 French Street
New Brunswick, NJ 08901

Dear Dr. Gallo:

Attached please find progress report for our project, “Brain Maturation and Self-Representation in Young Children with Autism Spectrum Disorders.”

The report consists of a narrative as well as financial report.

1. We are requesting that the balance of remaining funds from year 1 carryover to year 2.

The amount reflects an amount over 25% due to the following:

There was a delay in lining up subjects as well as a delay in final IRB approval. IRB was approved on 6/22/07, with an effective date of 7/2/07.

Sincerely,

Michael Lewis, PI
Progress Report - New Jersey Governor’s Council on Autism

February 29, 2008

Three projects were completed this past year.
   1. A paper was submitted for review
   2. A symposium as organized for presentation
   3. A second paper is in preparation for submission

1. We submitted a paper to the journal *Developmental Psychology*
   ‘Self Representation and Brain Development’
   Michael Lewis and Dennis P. Carmody

Submitted November 19, 2007

Abstract
Two cross-sectional studies examined the relation between self representation and brain development in infants and young children. Self representation was assessed by mirror recognition, personal pronoun use, and pretend play. Structural brain images were obtained from magnetic resonance imaging. Brain development was assessed by a quantitative measure of maturation of temporo-parietal junction, temporal pole, medial frontal cortex, and occipital cortex. In the first study, 15 children (15 to 30 months of age; 3 females) without MRI abnormalities were assessed and in the second study 23 children (14 to 33 months of age; 11 females) with MRI findings were assessed. Only maturation of the left temporo-parietal junction was related to self representation after controlling for age. These findings provide evidence that brain maturation is related to the emergence of a representation of self in the human child.

2. We organized a symposium for presentation at the meetings of the International Society on Infant Studies. XVIth Biennial International Conference on Infant Studies
   Vancouver, Canada, March 27-29, 2008

The Emerging Self
Michael Lewis, Chair
Betty M. Repacholi and Andrew N. Meltzoff
Philippe Rochat and Claudia Passos-Ferreira
Dennis P. Carmody
Michael Lewis, Discussant
3. 'Self Representation in Autism' in preparation
Dennis P. Carmody and Michael Lewis

Abstract
Self representation in autism spectrum disorders was assessed using behavioral measures of mirror recognition, other-directed pretend play, and use of personal pronouns. Twenty children with autism spectrum disorder, ages 4- to 5-years, were evaluated for the severity of autism using the Autism Diagnostic Observation Scale (ADOS). Clinical evaluation diagnosed 11 children with autism disorder and nine with pervasive developmental disorder- not otherwise specified (PDD-NOS). The individual results of the three behavioral measures were aggregated to obtain an overall self representation score (SRS). The SRS score was highly associated with the scores on the ADOS subscales of socialization, stereotyped behaviors, and imaginative play. In addition, the SRS score differentiated between the diagnosis of autism disorder and pervasive developmental disorder – not otherwise specified. These results suggest that the simple behavioral measures of self representation, used by paraprofessionals, may be useful as a screening tool for signs of ASD that are applicable to nonverbal children.

Table. The association between autism diagnosis and self representation

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<th>Showing Other-Directed Pretend Play</th>
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"Brain Maturation and Self-Representation in Young Children with Autism Spectrum Disorders"

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| Video tapes, 20 year @$5, $100   | 600    | 0      | 600      |         |                                  |
| toys $200 1st year,            | 100    | 0      | 100      |         |                                  |
| miscellaneous $700              |        |        |          |         |                                  |
| **Other:**               | 14,520 | 0      | 14,520   |         |                                  |
| Imaging, 40ss*$600@ 20 per year|        |        |          |         |                                  |
| Post Doc Health/Life Insurance per year | 12,000 | 0      | 12,000   |         |                                  |

| Consultant          |        |        |          |         |                                  |
| Alan Leslie, Ph.D.  | 10%    | 0      |          |         |                                  |
| Steve Kairys, M.D.  |        |        |          |         |                                  |
| Travel              | 775    | 775    | 0        |         |                                  |

| TOTAL               | 145,876| 93,084 | 52,792   | 36% Carryover |
As outlined in our initial application, autism spectrum disorder (ASD) presents a constellation of symptoms that appear to emerge from developmental patterning abnormalities. These morphological changes may underlie the altered social interactions that characterize this condition. We proposed to determine whether several mouse behaviors currently thought to reflect core characteristics of human autistic symptoms are altered in several strains of opioid system gene targeted mice.

During the first year of funding, we successfully introduced into our laboratory the assay for social approach behaviors. Using male wild type C57/Bl6 mice of either 6 or 8 weeks of age (n=25 for each age), we obtain results confirming that the mouse prefers exploration of a novel mouse compared to an empty chamber (p<0.05 Time spent in empty chamber vs Time spent in mouse chamber). In addition, number of entries to each side was equal demonstrating that locomotion and exploratory behavior was equal. Initial findings suggest that social preference, as measured by the amount of time spent on each side, is equivalent in mice at both ages. Exploratory behavior, as measured by the number of entries to each side, is altered by age. Eight week old mice enter each side of the chamber an average of 9 times during the test phase whereas, six week old mice only enter each side of the chamber an average of 7 times (p<0.005 six weeks vs eight weeks).

We have also tested mice on the 129S6 background though the levels of exploratory behavior are low. For example, in the 10 minute test phase, male 129S6 mice enter each chamber an average of only 1-2 times, which makes it difficult to produce a valid determination of social preference. At present we are trying to identify modifications to the test or apparatus (such as an increase in chamber opening) that would increase the exploratory activity of the 129S6 strain.

Since we established that wild type C57/Bl6 mice respond in a reliable, quantifiable pattern to the social approach test, we have begun to examine the effect of opioid system mutation on social approach behaviors in mutant mice maintained on this background. Initial testing of one cohort of triple opioid receptor knockout mice at either 6 or 8 weeks of age (n=10 for each age) demonstrated that mutant mice still significantly prefer the chamber containing the novel mouse to the empty chamber (p<0.05 Time spent in empty chamber vs Time spent in mouse chamber). However, at six weeks of age triple opioid knockout mice have significantly fewer entries than age-matched wild type mice (p<0.05 Wild type vs Triple opioid knockout). By eight weeks of age, triple opioid knockout mice are no longer significantly lower in number of entries compared to wild type mice, although a trend is still present. In addition, the difference in exploratory behavior observed in wild type mice between mice age 6 weeks and 8 weeks also occurs in triple opioid knockout mice. We have also performed preliminary studies of individual opioid receptor knockout mice in this assay as proposed. Similar to wild type and triple opioid receptor knockout mice, male DOR-1 (n=3) and MOR-1 KO mice (n=5) show preference for the chamber containing the novel mouse. Likewise, age differences in exploratory behavior, observed in wild type mice, also occur in both mutant strains. However, the genotype specific decrease in entries observed in six week old triple opioid receptor knockout mice appears to segregate to the MOR-1 KO strain exclusively. In conclusion, while initial studies indicate that mutation of the opioid system does not alter social approach behaviors as measured by the time spent in the chamber containing a novel mouse, though this need to be confirmed in a second cohort. We did find reduction in exploratory behavior in mutant mice at six weeks of age, as measured by the number of entries into the two chambers that appears to be entirely due to mutation of the MOR-1 gene. Future studies will determine if the genotype-specific decrease in entries at 6 weeks is due to elevated anxiety regarding a novel environment in MOR-1 KO mice. We will
also introduce a “second-phase” social interaction paradigm in which the response to a second mouse by wild-type and opioid receptor deficient mice will be measured. Studies are also currently ongoing to identify whether social novelty interactions are also altered in opioid system knockout mice. Finally, we have begun to assemble material for the neonatal olfactory learning paradigm, which will constitute a large effort in year 2.

Budget expenditures. As of February 27, 2008, expenditures from year 1 of this grant total ~135K, including encumbered funds that are expected to be charged to the current year budget. We thus anticipate a $15K carryover (10%), though the official report with the final expenditures will not be available for three months after the closing date of Feb. 29.
New Jersey Governor's Council on Autism – 1st year status report
PI: Gleb P. Shumyatsky

We would like to submit a first-year status report for the grant award that we received from the New Jersey Governor’s Council on Autism.

We had the following Aims in the original application:
1. Study affiliative maternal behavior and adult social interactions in mice with elimination of GRPergic cells in the amygdala and in mice with lesions of the basolateral amygdala (BLA).
2. Study how risk and threat affect affiliative maternal behavior and adult social interactions in mice with GRP KO, GRPR KO and Statmin KO. These genes are enriched in the basolateral amygdala.

We worked mostly on Statmin knockout (STKO) mice and mice with lesions in the basolateral amygdala where we proposed study maternal affiliative behavior and social interactions. We strongly feel that we were very successful (and lucky) during the 1st year because we were able to make very interesting and provocative observations. The following is an update of what was done since the submission of the application.

1. We found that BLA lesions reduce pup retrieval behavior similar to STKO (Figs. S1A-B), which strongly supports our hypothesis that BLA is involved in pup retrieval.
2. We found deficits in pup retrieval in postpartum STKO mice (Figs. S1C-D). Number of pups born and survival rate were significantly lower for litters from STKO females (data not shown). Thus, both naive and postpartum STKO females have deficits in pup retrieval.
3. In contrast to the immediate rescue of the pup retrieval deficit (Fig. S1E), rescue does not occur if pup retrieval tested 1 hr following pre-exposure to pups (Fig. S1F).
4. Both in the open field (Fig. S2A) and elevated plus maze (Fig. S2B), STKO mice and mice with BLA lesions show deficiency in anxiety similar to what we previously published in STKO males.
5. Remarkably, in contrast to deficits in pup retrieval, STKO females show an enhancement in social interactions towards other females (Fig. S2C).
6. BLA lesions in WT females also led to enhancement in social interactions towards females (Fig. S2C). This strongly supports our hypothesis that BLA is involved in social behaviors.
7. We have found that STKO mice have normal organization skills as tested in hoarding paradigm (Fig. S2D). Also, motivation and depression in Porsolt swim test was normal in STKO (Fig. S2E). Thus, except for the BLA-dependent behaviors STKO are otherwise normal.
8. We have analyzed brain activity in STKO mice using c-fos and oxytocin staining. We have found that c-fos is induced significantly less both in LA and BA (which compose BLA) in STKO mice after pup exposure (Fig. S2F-G). We have found no abnormality in oxytocin function either in MPA or PVN.
9. Importantly, this award allowed us to collect enough data to submit a new R01 grant application to the NIH.
10. We now have a manuscript under review entitled “Statmin reveals opposing roles of basolateral amygdala in affiliative and social behaviors”.

During the 2nd year of the award we plan to study GRP KO, GRPR KO and GRP-IL2R transgenic mice in the paradigms we used with STKO females. We will also examine males (males can perform pup retrieval – our unpublished data) of all transgenic/knockout lines in these behaviors because this will make our study more relevant to autism, which is much more prevalent in males. Because all these behavioral paradigms are now set up in our lab, we will have faster progress in the future.

Discussion:

We have made significant progress during the 1st year of the award and now have a manuscript under review entitled “Statmin reveals opposing roles of basolateral amygdala in affiliative and social behaviors”. We are very excited about this work where a combination of the statmin knockout in mice and amygdala lesions unraveled contrasting differences in how the amygdala is required for strengthening parental affiliative behaviors and at the same time inhibiting adult social interactions. We strongly believe that this study will provide important information about the new dual role of amygdala-associated neural circuits in social and affiliative behaviors and look forward to the possibility of applying our results for autism translational research in the future.

A major characteristic of this proposal is that it represents a new direction in our research. Importantly, this new direction is solidly based on our earlier research program where we study the role of genes enriched in the basolateral amygdala (BLA) in learned and innate fear. Now, after we have described the role in fear of these BLA-enriched genes in great detail, we can use this knowledge as a basis to study the molecular mechanisms underlying amygdala role in risk assessment in relationship to social, parental and other innate behaviors. Our proposal has a strong interdisciplinary approach: we combine molecular biology, mouse genetics (knockout and transgenics), anatomic (lesions), neural circuitry (tracing studies) and behavior experiments to ask questions pertinent to this application. Equally important, the theoretical framework to our application is strongly based on clinical questions arisen from the work by the Amaral, Damasio and other laboratories that study the role fear in social interactions and risk assessment in primates and humans. Moreover, our new results, showing that statmin knockout (STKO) females and females with BLA lesions have enhanced social interactions, provide direct genetic and anatomic support to the Amaral’s hypothesis that the amygdala modulates social interactions as a danger detector. However, human and primate studies can rarely go into depth into the role of genes in these behaviors. This is why our approach opens up a new avenue for studying genetics of innate behaviors that are dependent on risk assessment.
Fig. S1. Affiliative maternal behavior in STKO females and in WT females with BLA lesions. (A and B) Virgin STKO females (WT=22, KO=21) and wildtype females with BLA lesions (sham=8, BLA lesion=12) show (A) deficiency in pup retrieval on day one and (B) day two; 1st, 2nd and 3rd, retrieval of the first, second and third pups. To better illustrate the differences (see insets in panels A and B), we averaged retrieval time for all three pups: there is significant difference between the control group and the STKO group ($P < 0.001$ for both days) as well as between the control group and the BLA-lesion group ($P < 0.05$ for day one). (C and D) Pup retrieval is deficient in postpartum STKO females (WT=25, KO=25) both for the first (C) and second (D) days. Inset in (C) shows the retrieval data for the first day using a smaller scale. (E and F) The pup retrieval deficit can be rescued by placing pups for five minutes in the nest of a virgin STKO female if (E) the female is tested immediately (WT=7, KO=10) but (F) not after a one hour delay (WT=11, KO=11). Results are presented as mean ± SEM.
Fig. S2. (A and B) Innate fear is deficient in STKO females and in BLA-lesioned WT females. In (A) the open field the mutants (WT=10, KO=10) and lesioned animals (sham=10, BLA lesion=12) spend more time in the center of the arena and in (B) the elevated plus maze the mutants (WT=11, KO=11) and lesioned (sham=9, BLA lesion=12) animals spent more time in the open arm compared to control mice. * represents $P < 0.05$, *** represents $P < 0.001$. (C) Social recognition is enhanced in STKO mice (WT=10, KO=9) and in WT females with BLA lesions (sham=10, BLA lesion=10). ** represents $P < 0.01$, *** represents $P < 0.001$. (D) Organizational skills as tested by hoarding behavior are normal in STKO females (WT=8, KO=8). (E) The general level of motivation and depression as analyzed in Porsolt forced swim test is normal in STKO females (WT=10, KO=13). Results are presented as mean ± SEM. (F and G) During pup retrieval Fos is induced significantly less in the lateral and basal amygdala nuclei in STKO females (PR=8, Naive=4) compared to WT mice (PR=8, Naive=4). * represents $P < 0.05$; ** represents $P < 0.01$. PR, pup retrieval. Results are presented as mean ± SEM.
PROGRESS REPORT
SUBMITTED TO: NEW JERSEY GOVERNOR'S COUNCIL ON AUTISM

OXIDATIVE STRESS AND BRAIN METABOLISM IN AUTISM


T.P. Stein, PhD
Professor of Surgery, UMDNJ-SOM
Science Center 110A
40 East Laurel Road
Stratford NJ 08084

e-mail: tpstein@umdnj.edu
phone: 856-566-6036
fax: 856-566-6040
PROGRESS REPORT: SCIENCE

OBJECTIVES

1. To develop isotope dilution gc-ms assays for the isoprostane metabolites, 2,3 Dinor-5,6 dihydro-PGF$_2$ and iPf$_{4\alpha}$-VI. 2,3 Dinor-5,6 dihydro-PGF$_2$ is derived from Arachidonic acid (AA), an $\omega$-6 polyunsaturated fatty acid (PUFA). iPf$_{4\alpha}$-VI is a derived from the oxidation of two $\omega$3 PUFAs, eicosapentaenoic (EPA) and Docosahexaenoic (DHA) acids. DHA is the major fatty acid in brain. Accomplishment of this objective first requires the synthesis of the above metabolites with and without deuterium labels.

2. To show that the excretion of both 2,3 Dinor-5,6 dihydro-PGF$_2$ and iPf$_{4\alpha}$-VI are increased with autism. Accomplishment of this task requires the collection of a series (n=40) urines from autistic and control children.

3. To develop and use assays for the DHA derived resolvins D$_2$, D$_4$, D$_5$ and D$_6$ and neuroprotectin. Accomplishment of this task requires the completion of the syntheses described in objective #1 and then assay development.

OBJECTIVE #1. ORGANIC SYNTHESSES.

1. Synthesis of 2,3 Dinor-5,6 dihydro-F$_2$-isoprostane (F$_2$-Isop-M)

The synthesis of the isoprostane urinary metabolite, 2,3 Dinor-5,6 dihydro-F$_2$-isoprostane (F$_2$-Isop-M) (37), has been accomplished as outlined below using our previously published methodology (42, 44, 45) (figure 1). Using the deuterated intermediates and following the same sequence we achieved the synthesis of the deuterated urinary isoprostane metabolite. All compounds synthesized have been checked for purity by $^1$H-NMR, $^{13}$C-NMR, UV, FT-IR and HPLC-MS.

Figure 1. Scheme for the synthesis of 2,3 dinor-5,6 Dihydro-F$_2$-isoprostane

Reagents and conditions: (a) t-BuLi, CuCN, MeLi, Et$_3$O, -78 °C / methyl acetoacetate, Et$_3$O, -78 °C to rt, CH$_3$COOH; (b) LiBH$_4$, THF, -40 °C; (c) HF/Py, THF, 0 °C; (d) LiOH, THF/H$_2$O, 0 °C, H$^+$
2. Synthesis of iP$_{4\alpha}$-VI (Neuroprostane urinary DHA/ EPA metabolite)

Reagents and conditions: (a) BuLi, CuCN, MeLi, Et$_2$O, -78 °C / methyl acetoacetate, Et$_2$O, -78 °C to rt. CH$_3$COOH; (b) LiBH$_4$, THF, -40 °C; (c) HF/Py, THF, 0 °C; (d) LiOH, THF/H$_2$O, 0 °C, H$^+$

Figure 2. Scheme for the synthesis of iP$_{4\alpha}$-VI

The synthesis of the urinary DHA / ERA metabolite is now in the last step awaiting the two component coupling in both natural and deuterated form. The deuterated intermediates have been prepared for the final synthetic step.

2. Synthesis of d2-Resolvin D2

As described in the original application we already had completed the synthesis of Resolvin D2 and Resolvin D5 (40, 41). To make the deuterium analogs we used the same strategy but employing $^2$H$_2$O / $^2$H$_3$O$^+$ in the presence of Zn/Cu/Ag to introduce the deuterium isotopes in the molecule. All compounds synthesized have been checked for purity by $^1$H-NMR, $^{13}$C-NMR, UV, FT-IR and HPLC-MS.
OBJECTIVE #2. CLINICAL SAMPLE COLLECTION

Completion of objective #2 requires that samples from a suitable population of children be available for analysis. Rather than just collect random urines from autistic and control children as originally proposed we modified the clinical protocol in order to strengthen the statistical power of our analyses. Briefly we are arguing that the production of AA, EPA and in particular DHA metabolites is perturbed in some cases of autism. The anomalies should show up in the urinary excretion of these metabolites. If the metabolic pathway is perturbed, giving a challenge of DHA may accentuate the perturbation. Hence we modified the clinical protocol to collect urine before and four weeks after DHA (200 mg) treatment in order to increase the chances of obtaining convincing data to support our hypotheses.

Newly diagnosed children with autism were recruited from the UMDNJ Autism Center. Healthy controls were recruited from the Pediatric Ambulatory Center, New Jersey Medical School, UMDNJ, Newark, NJ. All autistic children were newly diagnosed so that they are naïve to medication. As in our published study, the diagnosis of autism were confirmed by Autism Diagnostic Interview – Revised (ADI-R), Autism Diagnostic Observation Schedule-Generic (ADOS-G) in addition to DSM IV criteria. All subjects were be carefully screened for signs of infection or inter-current illness on the
day of specimen acquisition, subjects with acute illness were excluded or rescheduled for urine sampling when the subject recovered from the acute illness.

We have completed the collection of complete sample sets from 43 cases (43 sets of urine together with full clinical characterization plus 20 age matched controls). The target is 60 cases.

ASSAY DEVELOPMENT (SPECIFIC AIM #2) AND HYPOTHESIS TESTING (SPECIFIC AIM #3)

Development and completion of the assays require that the objectives listed above be completed. Therefore most of this work will be done during the second year of this project. However we have made a start, basically to evaluate various types of gc columns, gc conditions and evaluating potential ions. GC and column conditions are crucial for quality analyses since the compounds are very similar. Essentially we used the approach developed by Serhan (1) using a two step derivitization procedure, first esterification with diazomethane followed by acylation with N.O bis(trimethylsilyl)trifluoroacetamide using a 30 m C18 column. Some work is needed to improve the clean up of urine and the separation.

Reference.

FINANCIAL REPORT
OXIDATIVE STRESS AND BRAIN METABOLISM IN AUTISM
PI: T.P. STEIN, PhD, UMSNJ-SOM
March 10, 2008

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BALANCE

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FINANCIAL REPORT: NOTES:

This report covers the period May 1, 2007 to February 29, 2008 (10 months). The reason for the delayed start was that final IRB approval was not obtained until April 2007. The attached spreadsheet shows the total amount expended up till February 29, 2008 together with the projected amounts for March and April. The 10 month data is in italics; the 12 month estimate is in regular type.

1. Two departments (Cell Biology, UMDNJ-SOM for A. Rodriguez and Neurology UMDNJ-NJMS for the nurse) did not bill the grant appropriately. Funds were not transferred from the grant to relieve the departments' salary payments. Back-billing is not permissible. Therefore, this delay in cost transfer means more money for the research since the individuals were working on the project. I.e., due to delays in processing change of source of funds requests by the two departments the two departments ended up paying more these individuals' salaries than they should have. Hence the large carry over of $71965. We are requesting permission to carry over these funds into Year 2.

2. $15,500 is for the purchase of one (1) non-polar chiral column. This will speed up the organic syntheses by speeding up the purification process for intermediates and end product.
February 21, 2008

Kendell Sprott, MD, JD
Chair
Mike Gallo Jr., PhD
Director
New Jersey Governor's Council on Autism
UMDNJ-Child Health Institute
89 French Street
New Brunswick, NJ 08901

Dear Dr. Sprott and Dr. Gallo:

Attached is a first year progress report on our Governor’s Council on Autism funded project entitled “Understanding the scope of autism in New Jersey: Characteristics of children diagnosed with autism spectrum disorders by the age of 8 years, NJ, 2000-2002”. We include a summary of our progress on our goals, as well as a budget update.

Please let us know if you have questions or concerns.

Sincerely,

Pauline Thomas

Pauline Thomas, MD
Associate Professor.

cc: Walter Zahorodny, PhD, Pediatrics
William Halperin, MD, MPH, DrPH, Preventive Medicine & Community Health
William Pojedinec, Administrator, Ob/Gyn/Women’s Health

Introduction

With this project we are analyzing two years of population-based autism surveillance data collected under a CDC funded autism CDC funded autism surveillance project. Investigators in the NJMS Department of Pediatrics, led by Dr. Walter Zahorody, used methods developed by the CDC to identify all children meeting criteria for autistic spectrum disorder (ASD) in four counties of New Jersey. Criteria for ASD were determined from reviews of existing professional evaluations in health and special education records. In participating school districts, records were reviewed for all 8-year-old children registered for Special Education. Records in clinical sites specializing in developmental pediatrics and / or autism were also reviewed. Children were counted if they were born in 1992 or 1994, and lived in a participating district at age 8 (2000 for children born in 1992, 2002 for children born in 1994). Children were counted not only if the school or medical facility had diagnosed autism, but also if symptoms and signs abstracted from school and clinic based evaluations met criteria for possible autism, set by CDC. (Methods for the surveillance system can be found in MMWR, February 9, 2007 / 56(SS01); 1-11).

NJ ASD prevalence rates for 2000 and 2002 were previously reported by CDC and compared to ASD rates from fourteen other states in a CDC group. (MMWR, February 9, 2007 / 56(SS01); 1-11) Overall, in the CDC ASD surveillance network, the rate of autism was 1 in 150 children nationwide, but in NJ the rate was higher, at 1 in 100 children.

In our project we have the opportunity to determine factors associated with autism in the four NJ counties, and to begin to examine hypotheses as to why NJ had the highest rate in the CDC ASD surveillance network.

Goals

We proposed the following goals for our project:


In our application we proposed to test the following hypotheses:

1) In NJ, there is no difference in rate of ASD between white non-Hispanic, black non-Hispanic, and Hispanic children.

2) In NJ, there is no difference in rate of ASD by SES of the school district or the family.

3) Paternal age is associated with rate of occurrence of autism diagnosis in New Jersey.

With funding from the NJ Governor’s Council on Autism, we are testing these hypotheses, by analyzing two years of data collected by the NJAS surveillance project.

**Progress**

Preliminary analyses to examine the three hypotheses are completed. For hypotheses 1 and 2, we have completed analysis for the 1992 birth cohort. For hypothesis 3, we have used a combined data set of the 1992 and 1994 birth cohorts to examine paternal and maternal age, for the subset of children for whom that data is available.

**Summary of findings** We have begun analysis for two hypotheses:

Hypothesis 1: In NJ, there is no difference in rate of ASD between white non-Hispanic, black non-Hispanic, and Hispanic children.

Hypothesis 2: In NJ, there is no difference in rate of ASD by SES of the school district or the family.

In the 1992 birth cohort of children, whose educational and medical records were reviewed as of 2000 when they were 8 years old, there were 29,714 children in 78 participating school districts, of whom 295 were found to have an autism diagnosis or signs and symptoms consistent with an autism diagnosis. Approximately 70% of the children met criteria for the classic condition Autistic Disorder, and 30% met criteria for the portion of Autism Spectrum Disorder (ASD), not

otherwise specified (NOS). A manuscript of these findings has been prepared for submission to the journal Pediatrics. Submission is anticipated for March 2008. (When the manuscript has been accepted, we will share it with the Governor’s Council. If any member of the Governor’s Council would like to see a draft before that time, please contact the PI, Dr. Thomas.)

While the crude rate of autism was higher in whites and blacks and lower in Hispanic children, the confidence intervals for these differences overlapped, and the differences were not significantly different. Although analysis of the 2002 data has not been completed, we have looked at crude rates by race/ethnicity. The findings are slightly different, with black children having the lowest rate, but again not significantly different from the rate in white and Hispanic children.

There is a significant association of autism diagnosis with the wealth of the school district as measured by District Factor Group (DFG). DFG is a six-factor SES index, designed and implemented in New Jersey and used since 1974 to provide the basis for analyzing the contribution of socioeconomic status on educational outcomes. The rate of ASD among children enrolled in school in the wealthiest districts was significantly higher than the rate in the poorest districts. Also, children in wealthier school districts had higher numbers of professional evaluations than other children.

Our co-investigator, Bo Peng, biostatistician, is exploring this association in more detail.

Summary of progress on third hypothesis

3) The third analysis we intend is to determine whether paternal age is associated with rate of occurrence of autism diagnosis in New Jersey.

To evaluate this hypothesis, we are comparing birth certificate data from children with ASD in our surveillance cohort (birth years 1992 and 1994), with birth certificate data from other NJ children born in the same census tracts in the same two years. This analysis is led by Dr. Katherine Hempstead, Director, Center for Health Statistics, NJDHSS and Assistant Research

Professor, Rutgers University. Dr. Hempstead has compiled data from the birth certificates, and preliminary analysis shows no marked association of ASD with either maternal or paternal age. However, multivariable analysis and separation of children by type of ASD diagnosis (Autism disorder vs. PDD NOS) has not been completed. Ms. Bo Peng will assist with the analysis, pending IRB approval from NJDHSS for release of non-identified birth certificate data.

A proposal for a poster presentation on the findings has been accepted for the annual meeting of the Population Association of America, scheduled for April.

Other analyses

A final decision on additional analyses will be made in March 2008. Topics under consideration for analysis in year 2:

1. Complete 2 papers from year 1 and submit to peer reviewed journals:
   a. 1992 birth cohort
   b. Association of paternal and maternal age with ASD in NJ

2. Descriptive analysis of 1994 birth cohort

3. Combine information from the two birth cohorts to analyze the association of socioeconomic status of both the school district (DFG), SES of the census block of the residence of the child at age 8 (2000 and 2002), and for children for whom a matching birth certificate is available, using education status of either or both parents (a proxy for SES).

4. A descriptive analysis of psychoactive medications prescribed to our two cohorts of 8 year olds. Analysis will include possible associations between sex, race, SES and ASD type.

5. Time permitting, NJ-specific findings will be compared to data from the pooled ADDM network.

Our group will also cooperate with another Governor’s Council on Autism funded project, PI Dr. Wartenburg, to evaluate the role of air and water contaminants in the area where the children were born.

Further analyses will be considered pending findings from analyses currently underway, and pending advice from our scientific advisory committee.

**Other Progress**

**Development of an ASD severity index.**

Dr. Zahorodny, Ms. Peng, and Dr. Thomas with input from Dr. Hempstead developed a “severity index” using data available in the NJ ASD surveillance system. The index uses: Global severity, assigned by expert clinicians who reviewed the data abstracted on each case; and number of Autism Discriminators. The Index is as follows:

- **Severe:**
  - Severe global impairment *or*
  - Above the median number of Autism Discriminators (>3) *or*
  - Any other impairment (vision, hearing, seizures) *or*
  - IQ < 70

- **Moderate:**
  - Moderate global impairment *and*
  - Median or lower number of autism discriminators *and*
  - No other impairment (vision, hearing, seizures)

- **Mild:**
  - None of the above: *i.e.*,
    - Mild impairment from autism, *and*
    - Fewer than the median number of autism discriminators *and*

- No other impairment (vision, hearing, seizures)

We had intended to include IQ at each level in the severity index, but this is not possible as there is a bias in which children have an IQ recorded. More children in the wealthier school districts have an IQ recorded. We will use very low IQ in same way as other impairment.

Because CDC had not required expert clinician review of case information from the 1994 birth cohort, there were approximately 170 cases that needed additional expert review. Dr. Audrey Mars and Ms. Mildred Waale, two experienced NJAS expert reviewers, were engaged to review these cases, allowing us to assign a global severity index and number of autism discriminators to each child. These data have been entered onto an excel spreadsheet and will be analyzed by Ms. Peng for stratified analysis by ASD severity.

Scientific Advisory Committee

We have had two meetings with our scientific advisory committee, and plan for 3 more meetings during the final grant year.

The Scientific Advisory Committee members are:

- Michael Brimacombe, PhD, Associate Professor, New Jersey Medical School (NJMS) Department of Preventive Medicine and Community Health (DPMCH)
- Charles Cartwright, MD, Director of the Autism Center at NJMS.
- Franklin Desposito, MD, Professor, NJMS Department of Pediatrics
- William Halperin, MD, MPH, DrPH, Professor and Chair, NJMS DPMCH, and Chair, UMDNJ School of Public Health Department of Quantitative Methods
- Katherine Hempstead, PhD, Director, New Jersey Department of Health and Senior Services (NJDHSS) Department of Vital Statistics (Also a co-investigator)
- Soyeon Kim, PhD, Assistant Professor, NJMS DPMCH
- Catherine Rice, PhD, Director, Autism Epidemiology Studies [CHECK TITLE], Centers for Disease Control and Prevention.
Budget update:

As of February 29, 2008, we will have spent $106,376.00. We are in process of purchasing supplies at $2,315.00, and will pay $685.00 for Dr. Hempstead to travel to present her paper, including conference registration.

As of March 1, 2008, we will have remaining $3,829.00 of the $113,205.00 awarded for Year 1.

We request permission to carry over the $3,829.00.

Please let me know what other information you require.

Submitted by Pauline Thomas, MD, February 21, 2008.
First-year Status Report

Project Title: Protection against early toxicant exposure in a mouse model of autism

Principal Investigator: George C. Wagner, Ph.D.

Title: Professor

Institution and Department: Rutgers University, Psychology

Address: 152 Frelinghuysen Rd., Busch Campus, Piscataway, NJ 08854

Phone: 732-445-4660

Fax: 732-445-2263

E-mail: gcwagner@rci.rutgers.edu

Financial Statement: This was sent directly to Dr. Michael Gallo by Mr. Frank Cotchen of Rutgers University Office of Research and Sponsored Programs.

Abstract: We have developed a novel animal model of autism which has proven to be sensitive, allowing us to categorize toxicant-induced neurobehavioral deficits along a developmental timeline and, independently, to detect effects of gene alteration on these same neurodevelopmental measures. In addition, we demonstrated that antioxidant pretreatment protects genetically-intact mice from toxicant-induced neurobehavioral deficits. Major limitations of this work, to date, have been the absence of morphological correlates of the toxicant-induced behavioral disruption and, of course, our failure to demonstrate that our antioxidant treatment is capable of protecting mice against such morphological damage induced by toxicants. Finally, we have yet to administer any of toxicants we have studied (metals, drugs, pesticides) to genetically-altered subjects. Accordingly, we now propose to administer our standard toxicant, valproic acid (VPA), to mice with altered expression of Engrailed2 (a gene involved in cerebellar development and highly associated with autism) and to assess these mice for deficits in neurobehavioral development as well as for morphological evidence of cerebellar cell damage. We then plan to prevent the occurrence of these toxicant-induced deficits by administration of Trolox, a water soluble derivative of vitamin E. We anticipate that En2−/− mice will be more sensitive to disruption of behavioral development and manifest more cerebellar cell damage than their wild-type controls and that Trolox administration will protect mice on both measures. These observations would have important ramifications for autism, known to be associated with alterations in Engrailed expression and cerebellar damage and thought to be triggered by early toxicant exposure.

Specific Aim 1: Demonstrate that Engrailed2-mutant (En2−/− and En2+/−) mice are more sensitive to the neurobehavioral and morphological damage induced by VPA than their wild-type (En2+/+) controls;

Specific Aim 2: Determine if pretreatment with Trolox protects mice (of all three genotypes) against the neurobehavioral and morphological damage induced by VPA.

First-Year Progress Report:
As noted in our original proposal, the most serious deficiency with our animal model was that we had no morphological markers of VPA-induced damage. Much of our first year effort was dedicated to achieving such an endpoint and we have accomplished this with a manuscript that is
now “in print” and attached (Yochum et al., in print). As stated in the original proposal, we targeted the cerebellum as damage to that region has been associated with human autism. We used our standard VPA dose and treatment time (400 mg/kg on P14) and elected to use TUNEL stain for neuronal apoptosis as our morphological marker. As predicted, the cerebellum was affected by the VPA and we fully characterized the time course for the damage. We examined other regions and, somewhat to our surprise, we observed hippocampal damage as well. The hippocampus is a second region known to be affected in human autism. Our manuscript fully characterizes the time course of the VPA-induced damage in both regions. Embedded in this effort was a second, rather tedious task, of learning how to quantify the cell counts. The details of the NIH-SCION cell counting and statistical analyses are in the manuscript and mentioned here as a first-year accomplishment that was not part of our initial objective.

Our second major objective is to demonstrate that antioxidants protect the mice against the toxicant-induced damage. We have already accomplished much of our stated objective as detailed in our recent publication, also attached (Ming et al., 2008). Here we demonstrated that Trolox and vitamin E protect mice against MeHg and VPA respectively. A portion of the former data (i.e. that Trolox protects against MeHg) had served as Preliminary Data for our original proposal. That study was completed and presented together with the newer data on vitamin E and VPA. Collectively, these data were interpreted as follows: autism may the result of early exposure to any of a number of environmental toxicants acting upon genetically-sensitive individuals. A common feature of the toxicants is their capacity to engender neuronal oxidative stress and, therefore, protection may be afforded by pretreatment with antioxidants. The online availability of this second publication generated sufficient interest even before its formal publication. As a result we will now be writing an invited chapter on this topic in a forth-coming text.

The last objective of our original proposal was initiated late last year. That is, to combine the above observations in genetically-altered mice. Our first attempt revealed a bit of an unexpected result, namely that the c57/129 background strain of our Engrailed2/ko mice (i.e. wild-type mice) are far more sensitive to the VPA than our first strain (BALB/c). Too many cells were affected, a finding that will spawn a separate strain study with the prediction that the behavioral deficits will also be more severe in this strain. In any case, it was not possible to detect genotypic differences and so we will conduct a full dose-response curve for the apoptotic effects of VPA in the three genotypes (now underway). The last step would be to protect these mice with our antioxidants - our primary objective for year 2.

Late in year 1 we acquired a second genetically-altered strain, GSTM1/ko mice, now being bred in the lab. GST polymorphisms were identified in mothers of children with autism (Williams et al., Archives of Pediatric and Adolescent Medicine, 2007, 161, 356-61). Thus, this new ko strain appears to be more relevant than our original selection in that it directly links a deficit in maternal response to oxidative stress to autism. We predict that these mice will be more sensitive to the same toxicants already tested and, during year 2, these studies will be conducted in parallel to those on the Engrailed2/ko mice.

February 29, 2008

Mike Gallo Jr., Ph.D.
Director
New Jersey Governor's Council on Autism
UMDNJ-Child Health Institute
89 French Street
New Brunswick, NJ 08901

Dear Mike:

Enclosed please find the first year status report for our Autism project. I just noticed that you also want a financial, but am not sure what that should look like. I tried calling you but no one was in your office.

Hopefully, we can touch base early next week and sort this out. When I send in the financial report, I will include a request for carryover. We were unable to spend a lot of our funds because it took several months to hire appropriate personnel.

Thanks.

[Signature]
Goals:
The primary goals of this project are: (1) to describe the socio-demographic, spatial and temporal prevalence patterns of Autism Spectrum Disorder (ASD) in four counties of New Jersey (Union, Essex, Hudson and Ocean) and (2) to assess the association of these prevalences with levels of contaminants in the air (e.g., hazardous air pollutants (HAPs)) and drinking water (e.g., trihalomethanes (THMs)). The descriptive portion of this project is designed to provide a context for the analytic studies. The analytic studies are designed to replicate and extend work done elsewhere (e.g., HAPS\(^1\)) and to investigate an hypothesis raised by ATSDR staff\(^2\).

Objectives for Year 1 (3/1/07-2/29/08):

1. **Obtain IRB approvals**

   **Activities:** Complete the necessary paperwork to submit the application to the relevant agencies.

   **Status:** Ongoing.

   **Discussion:**
   - **RWJMS IRB**  Our study was approved on 1/18/07.
   - **NJMS IRB**  Dr. Zaharodny submitted an amendment to add us to his approved study and this was approved on 10/31/07.
   - **NJDHSS IRB**  We have encountered some delays in obtaining IRB approval from the New Jersey Department of Health and Senior Services (NJDHSS). We contacted the Center for Health Statistics (CHS) to request a copy of Dr. Zaharodny’s linked ASD-birth certificate file that they had already created for a separate study along with other files necessary to undertake our study. Kathy Hempstead, Director of the CHS, suggested that once we get IRB approval, instead of requesting the ASD data ourselves we should submit all the other data (e.g., exposures, other risk factors and confounding variables) to CHS and they would perform the requisite analyses for us, overcoming any confidentiality concerns. However, as the next step in the IRB process, we contacted Matt Weinberg, MB at the NJDHSS IRB office who provided some advice for the protocol, suggested that we should do the analyses ourselves, and that NJDHSS may be able to provide us with the data we need to do so. Currently he is reviewing our protocol prior to formal submission to the NJDHSS IRB.

2. **Hire Staff**

   **Activities:** Identify a senior researcher to manage the project and a research assistant to help with the analytic work and the day-to-day operations of the project.
Objectives for Year 1 (3/1/07-2/29/08):

Status: Ongoing.

Discussion: Dr. McWilliams, an experienced PhD epidemiologist was hired as an Assistant Professor in the Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School on 10/1/07. She will manage the project.

Currently, we are seeking a research assistant with strong quantitative skills.

3. Develop Detailed Study Protocol

Activities: The Protocol was written and data requirements were determined based on examination of the available exposure data, birth certificate data and case data.

Status: Ongoing.

Discussion: Revisions have been made based on IRB advice. After revision, the protocol was sent to Matt Weinberg at the NJ-DHHS IRB office on 2/12/08.

4. Data Acquisition

a. Acquire Census data
Activities: After online training, Dr. McWilliams downloaded 1990 and 2000 census data for the 4 NJ counties.
Status: Data acquired.

b. Acquire Air Toxics exposure data
Activities: Dr. McWilliams downloaded 1996 and 1999 US EPA National Air Toxics Assessment (NATA) Hazardous air pollutants (HAPS) data for the 4 counties in NJ.
Status: Data acquired.

c. Acquire water contaminant data
Activities: Drs. Wartenberg and McWilliams met with Steve Anderson from the NJ-DEP on 1/8/08 to discuss acquiring their 1990-2000 data on contaminants for areas served by the state’s public water supply utilities in the 4 counties.
Status: Ongoing.
Discussion: Dr. McWilliams will provide Dr. Anderson with a list of
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Daniel Wartenberg
Rita McWilliams
Walter Zahorodny

contaminants of interest other than trihalomethanes before March 1, 2008. Upon receipt of the list, Dr. Anderson will provide the data on THMs and select contaminants for areas served by the state’s public water supply utilities in the 4 counties.

Drs. Wartenberg and McWilliams had a conference call with Dr. Perry Cohn from the NJDHSS on 2/6/08 regarding his experience in acquiring data on contaminants for areas served by the state’s public water supply utilities. Dr. Cohn provided guidance via a document: Volatile Organic Chemical (VOC) Contamination in New Jersey Public Water Systems from 1978-1990.

d. Acquire NJ ASD case data and NJ birth certificate data
Activities: Drs. Wartenberg and McWilliams held several discussions with Dr. Zahorodny regarding acquisition of the case data. He told us to address the issue with Katherine Hempstead at the Center for Health Statistics since he had sent the data to them to be linked to birth certificate data.
Status: Ongoing
Discussion: We are in discussion with NJDHSS regarding the IRB approval required for obtaining this data. Once NJ-DHHS IRB approval has been obtained, a letter requesting specific data will be sent to the CHS.

Objectives for Year 2 (3/1/08-2/29/09):

1. Finalize NJDHSS IRB approval

2. Organize data, review data quality and completeness and conduct descriptive analyses
   a. US Census data (sociodemographic parameters)
   b. Exposure data (air—HAPS; drinking water—THMs)
   c. Birth data (parental risk factors, pregnancy and birth complications)
   d. Cases (Autism descriptors)


4. Assess the possible association of exposure data and ASD, adjusting for confounding variables and effect measure modification.
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Daniel Wartenberg
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Walter Zahorodny

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   a. US Census data (sociodemographic parameters)
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References Cited


Pediatrics Department  
185 South Orange Ave F 570  
Newark, New Jersey 07103  

February 21, 2008  

Dear Doctor Gallo: 

Attached is a first year progress report on New Jersey Autism Study: Population-Based Surveillance of Autism Spectrum Disorders in New Jersey, a public health investigation supported by the New Jersey Governor’s Council for Medical Research and Treatment of Autism. 

Included is a summary of aims and objectives, successes and setbacks, as well as a first year budget report. 

Please let me know any additional requests and any questions or comments. 

Thank you very much. 

Truly,  

Walter Zahorodny, Ph.D.  
Assistant Professor
First Year Progress Report

Introduction

Autism is a complex developmental disorder characterized by deficits in social and linguistic capacity and by restricted, anomalous or repetitive behavior. The epidemiology of autism is not established and remains a basis for continuing controversy. The current definition of autism regards it as a spectrum disorder. Since there is no biological test for Autism Spectrum Disorders (ASD) and since autism is a heterogeneous phenomenon, active ASD surveillance is essential for the scientific understanding and practical appreciation of this disorder. The New Jersey Autism Study (NJAS) has established baseline Autism Spectrum Disorders (ASD) prevalence estimates for two cohorts of eight-year old children. Findings from the initial NJAS investigation disclosed a high rate of autism (1%) and demonstrated that New Jersey is an ideal state for implementation of a multiple source ascertainment system (MMWR, 56; SS01).

Aims

The aims of the ongoing investigation, supported by the Governor's Council for Medical Research and Treatment of Autism, are to establish Autism Spectrum Disorders (ASD) prevalence estimates in New Jersey, by the proven, population-based, multiple source method, for 2006, and to describe the characteristics and distribution of ASD in the New Jersey metropolitan region. In doing so, this investigation will permit the comparison of ASD rates in our region across a six-year period and across demographic and geographic groups. By linking the ASD case information with other New Jersey datasets, like the New Jersey Birth Certificate Registry, this investigation may further the understanding of ASD types and risk factors. Since few studies have monitored trends in autism prevalence in a large, diverse, population, this investigation may have scientific value. Since the methods and definitions employed by the New Jersey Autism Study yield accurate and representative estimates of ASD prevalence and expression, findings from this project may be of significant value to public health and education authorities.

Surveillance Population and Method

The surveillance region surveyed by this project includes Hudson, Union, Essex and Ocean Counties. The cohort is limited to children born in 1998, residing in one of the surveillance counties, in 2006. The definitions, methods and procedures of the New Jersey Autism Study have been described in detail elsewhere (MMWR, 56; SS01) and were included in the project proposal. The New Jersey Autism Study is the bona fide agent of ASD surveillance in New
Jersey and is implemented under waiver of informed consent by the New Jersey Medical School Institutional Review Board (Protocol 0120010288).

**Objectives Addressed in Year One**

This project has made very good progress, to date. Many important pre-requisites were satisfied before the initiation of ASD ascertainment. These included: information-sharing and source agreements with 67 school districts and ten clinical centers/providers, letter of support from (New Jersey Department of Education) Commissioner Davy, recruitment, training and development of a professional research team, institutional (IRB) approval, including human subjects confidentiality certification of study personnel, implementation of security and confidentiality procedures, implementation of quality assurance and quality control procedures, data request to all cooperating sources. Case ascertainment was initiated once all researchers (abstractors) showed high reliability with the study procedures, including use of the database program and once the database security and data replication procedures were confirmed. Review and abstraction (ascertainment) activities started in July and continue to this date. As of February 21, 475 source records, representing approximately 4,000 professional evaluations have been reviewed for autism triggers. 135 cases were identified to have one or more autism trigger(s) and were fully abstracted, in accord with the study methods.

To date, review and abstraction (ascertainment) activities have been completed in twenty school districts and two clinical centers. This represents approximately one third of the likely total number of cases to be reviewed. Quality assurance and quality control measures were/are implemented regularly and indicate a high degree of abstractor reliability (> .90), consistent with the procedures of the New Jersey Autism Study (NJAS), the Centers for Disease Control and Prevention (CDC) and the Autism and Developmental Disabilities Monitoring (ADDM) Network for active, autism and developmental disabilities case-finding.

ASD case ascertainment is ongoing in New Jersey school districts and clinical centers. Data review requirements will be great for the next nine to ten months. Experts have been identified and are undergoing human subjects confidentiality certification, study briefing and training. It is expected that Expert Review activities for determination of ASD case status will begin in May or June. These activities will establish the number of 8 year olds in the surveillance region meeting criteria for an ASD and will lead to the description of the functional, demographic and functional characteristics of the cohort.
Discussion of Preliminary Findings, Successes and Setbacks

Multiple source autism (developmental disabilities) ascertainment activities are very labor-intensive and require a high degree of coordination. The investigator was able to establish a surveillance research team within three months and to implement all the necessary legal and privacy safeguards for study activities. The many necessary pre-conditions for public health surveillance were satisfied quickly, due to the experience of the investigator with this type of investigation and the high degree of support for ASD surveillance in New Jersey by health and education authorities. Review and abstraction activities have been well received and are being professionally implemented. Recently, in response to our request for cooperation with ASD surveillance, three school districts that did not participate with ASD surveillance for 2000 and 2002 decided that they would participate for 2006. The addition of these districts is welcome. The accuracy of prevalence estimates by the New Jersey Autism study can only improve with every additional source.

To date, preliminary evaluation of the number of cases for review and the number of professional evaluations for abstraction suggests that we are finding and, therefore, abstracting a larger than expected number of professional evaluations on a per child basis. For the first nine months of ASD (2006) surveillance, the abstraction (total number of professional evaluations) case-load was 35-40% higher than the abstraction case-load for surveillance in study years 2000 and 2002. The greater number of professional evaluations observed may be a reflection of wider or more frequent utilization of professional assessments for educational planning and/or health intervention, which may, in turn, be related to greater awareness of developmental disorders, like autism, by professionals and the public.

In any event, anticipation of a (35-40%) greater work (abstraction) requirement and the expectation of reviewing and abstracting records in three previously non-participating school districts, may be considered a setback. Even though more professional evaluations per child and more complete participation by sources is good news from the perspective of data quality, it made timely completion of study activities seem unlikely.

To address the setback to our schedule, to enhance the speed of abstraction, while preserving the data completeness, privacy and confidentiality, our study group developed a technological modification. This modification was systematically evaluated by the investigators and was found
to reduce the time required for abstraction by one third. Moreover, the technical modification maintains all data security elements and increases the accuracy of abstraction. Since the technical modification allows faster abstraction of data and accords more flexibility in scheduling field operations, it is now possible that ASD ascertainment and case determination, as well as specification of ASD prevalence for the study year will be completed by March 1 2009. While our technological modification will make for a great recovery, it is unlikely that there will be sufficient time to complete ASD trend analyses for the period 2000 to 2006 or to provide information about any trend in ASD prevalence before March 2009. Though we expect to have have established a link with the Birth Certificate Registry before the end of the study period, analysis of the linked data will occur afterwards.

Objectives for Year 2
Our objectives for year 2 are:
Completion of ASD case ascertainment activities at all cooperating sources,
Analysis of case information by independent experts for determination of ASD case status,
Maintenance of quality assurance and quality control procedures,
Data cleaning and preparation for analysis,
Specification of ASD prevalence estimates for the study population,
Description of ASD characteristics and the demographic distribution participate with ASD,
Development of link between the investigation and the Birth Certificate Registry.

Progress Relative to Stated Objectives
According to the objectives described in our application, to date, one objective has been achieved and activities toward achieving two other objectives are ongoing and on-schedule.

Objective 1: Recruitment and maintenance of the collaborative support necessary to accomplish population-based surveillance of ASD – achieved
Objective 2: Comprehensive review and abstraction of case information from multiple participating health and education sources – ongoing/on-schedule
Objective 4: Implementation of standard quality assurance and quality control procedures – ongoing/on-schedule

Similarly, five objectives stated in the application are pending and on-schedule:

Objective 3: Determination of ASD case status by independent reviewers
Objective 5: Specification of ASD prevalence estimates
Objective 6: Descriptive analysis of ASD case data
Objective 7: Other analyses
Objective 8: Link to New Jersey Birth Certificate Registry
Pediatrics Department
185 South Orange Ave F 570
Newark, New Jersey 07103

February 29, 2008

Dear Doctor Gallo:

Attached is a first year progress report on, Young Adults with Autism: A Pilot Epidemiologic Investigation, as supported by the Governor's Council for Medical Research and Treatment of Autism.

Included is a summary of aims and objectives, successes and setbacks, as well as a first year budget report.

Please let me know any additional requests and any questions or comments.

Thank you very much.

Sincerely,

Walter Zahorodny, Ph.D.
Assistant Professor
First Year Progress Report

Introduction
Autism is a complex developmental disorder characterized by deficits in social and linguistic capacity and by restricted, anomalous or repetitive behavior. The epidemiology of autism is not established and remains a basis for continuing controversy. Recent studies have shown higher than expected rates of autism among children in New Jersey and other states. While the descriptive epidemiology of autism in children is 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.

Using a thorough, population-based, surveillance methodology developed by the Centers for Disease Control and Prevention, the New Jersey Autism Study has established baseline Autism Spectrum Disorders (ASD) prevalence estimates for two cohorts of eight-year old children. Findings from this investigation disclosed a high rate of autism (1%) and demonstrated that New Jersey is an ideal state for implementation of a multiple source developmental disabilities ascertainment system (MMWR, 56; SS01).

Aims
The aims of this pilot study, sponsored by the Governor's Council for Medical Research and Treatment of Autism are: 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.

This pilot investigation is limited to persons born in 1988, residing in Union County in 2006. The ASD and other definitions, methods and procedures implemented by the New Jersey Autism Study have been described in detail elsewhere (MMWR, 56; SS01) and were included in the project proposal. The New Jersey Autism Study is the bona fide agent of ASD surveillance in New Jersey and is implemented under waiver of informed consent by the New Jersey Medical School Institutional Review Board (Protocol 0120010288).

Since almost no studies have ever sought to establish the prevalence of Autism Spectrum Disorders (ASD) among young adults and none have been attempted using modern
epidemiologic methods in a large, diverse, population, this investigation may have significant scientific value. Since the methods and definitions employed by the New Jersey Autism Study yield accurate and representative estimates of ASD prevalence and an objective description of the expression of ASD in many individuals, findings from this project may be of significant value to public health, public policy and education authorities.

Objectives Addressed in Year One
This project has made excellent progress, to date. Many important pre-requisites were satisfied before the initiation of ASD ascertainment activities. These included: information-sharing and source agreements with 21 school districts and 6 clinical centers/providers, letter of support from (New Jersey Department of Education) Commissioner Davy, recruitment, training and development of a professional research team, institutional (IRB) approval, including human subjects confidentiality certification of study personnel, implementation of security and confidentiality procedures, implementation of quality assurance and quality control procedures, data requests to all cooperating sources. ASD case ascertainment was initiated once all researchers (abstractors) showed high reliability with the study procedures, including use of the database program and once the database security and data replication procedures were confirmed. Review and abstraction (ascertainment) activities started in September and continue to this date. As of February 29, 375 source records, representing approximately 2,400 professional evaluations have been reviewed for autism triggers. Approximately 115 cases were identified to have one or more autism trigger(s) and were fully abstracted, in accord with the study methods.

To date, review and abstraction (ascertainment) activities have been completed in 15 school districts and one clinical centers. This represents approximately one half of the likely total number of cases to be reviewed. Quality assurance and quality control measures are implemented regularly and indicate a high degree of abstractor reliability (> .90), consistent with the procedures of the New Jersey Autism Study, the Centers for Disease Control and Prevention and the Autism and Developmental Disabilities Monitoring (ADDM) Network for active, autism and developmental disabilities case-finding.

ASD case ascertainment is ongoing in Union County school districts and clinical centers. Data review requirements will be great for the next six to nine months. Additional IRB requirements were satisfied with Hackensack University Medical Center. Approval of IRB modification at
Newark Beth Israel Hospital, on behalf of the investigation, is pending. Redundant information is provided to most potential clinical sources regarding the HIPPA exclusion for public health investigations, like this one. According to the study methodology, independent review and analysis of case information is accomplished by (developmental) experts, using DSM-IV-referenced diagnostic criteria. The professionals to serve as Experts for this study have been identified and are undergoing human subjects confidentiality certification, study briefing and training. It is expected that Expert Review activities for determination of ASD case status will begin in September. These activities will establish the number of 18-year olds in the surveillance region meeting criteria for an ASD and will lead to the description of the functional, demographic and functional characteristics of the cohort.

Discussion of Preliminary Findings, Successes and Setbacks

Multiple source autism (developmental disabilities) ascertainment activities are very labor-intensive and require a high degree of coordination. This project has enjoyed repeated successes during year 1. The investigator was able to establish a surveillance research team within three months and to implement the necessary legal and privacy safeguards for study activities. The many necessary pre-conditions for public health surveillance were satisfied quickly, due to the experience of the investigator with this type of investigation and the high degree of support for ASD surveillance in New Jersey by health and education authorities. This pilot project has been very well received by the Union County school districts and authorities. Agreements for participation are pending with with three private providers, one private hospital and the Division of Developmental Disabilities.

To date, preliminary evaluation of the number of cases for review and the number of professional evaluations for abstraction suggests that we are finding approximately 8 to 10 evaluations per subject, based on data (predominantly) from school sources. When ascertainment in Union County proceeds to the major clinical sources, additional evaluations per subject are likely.

No significant setbacks have been experienced.

Objectives for Year 2

Our objectives for year 2 are:

- completion of ASD case ascertainment activities at all cooperating sources,
analysis of case information by independent experts for determination of ASD cases
maintenance of quality assurance and quality control procedures,
data cleaning and preparation for analysis,
specification of ASD prevalence estimates for the (18-year old) study population,
description of ASD characteristics and the demographic distribution of the cohort,
establishment of a link with the New Jersey Birth Certificate Registry and
information-sharing with cooperating sources and sponsors.

**Progress Relative to Stated Objectives**

According to the objectives described in our application, to date, one objective has been
achieved and activities toward achieving two other objectives are ongoing and on-schedule:

- **Objective 1:** Recruitment and maintenance of the collaborative support necessary to
  accomplish population-based surveillance of ASD in young adults – achieved
- **Objective 2:** Comprehensive review and abstraction of case information from multiple
  participating Union County health and education sources – ongoing/on-schedule
- **Objective 4:** Implementation of standard quality assurance and quality control
  procedures – ongoing/on-schedule

Similarly, seven objectives stated in the application are pending and on-schedule:

- **Objective 3:** Determination of ASD case status by independent reviewers
- **Objective 5:** Specification of ASD prevalence estimates
- **Objective 6:** Descriptive analysis of ASD case data
- **Objective 7:** Other analysis
- **Objective 8:** Link to New Jersey Birth Certificate Registry
- **Objective 9:** Comparison of ASD rates and characteristics of 18-year old ASD cases and
  rates and characteristics of 8-year old ASD cases from Union County
- **Objective 10:** Informational outreach to communicate findings of the pilot investigation.

**Budget update:**

I project that as of, or before, April 30, 2008, this project will have spent approximately $90,000
of the allocated $118,899 for year 1. Therefore, I believe that the amount to carryover is less
than 25%. The full amount of funding is needed to maintain the current ascertainment and case
determination activities. I respectfully request the carryover of year 1 funds to year 2 of this
project.

Almost all of the year 1 funds to this project were personnel costs.
Scheduled funding for year 2 activities of this investigation is $84,729
Zalcman, Steven S. (PI)

New Jersey Governor's Council on Autism

PI: Steven S. Zalcman, PhD

Title: Treatments for the prevention of autistic-like behavior in offspring of mothers infected with influenza virus during pregnancy: An animal model

Year 1 Progress Report

Award start date: July 1, 2008

March 14, 2008
Progress Report:
The following describes work conducted during the current funding year that is of fundamental importance to our project, and outlines experiments and expenditures for the remaining portion of Year 1.

Behavioral Testing – Social Investigation: A core dimension of Autism Spectrum Disorder is an impairment in reciprocal social interactions. In mice, social investigation is studied by placing same-sex and experimental group pairs of mice in the center of a test arena. During the current year, we have made progress evaluating social investigation patterns in young adult Balb/c mice (the age at which Balb/c offspring of infected mothers are to be tested). We have also evaluated the effects of inflammatory substances involved in the host’s response to infection on these patterns. The 15-min test session was conducted under normal illumination, filmed by a VHS camera and behavior was scored at a later date. We recorded the latency (sec) to make initial contacts, as well as the number of contacts made and the duration (sec) of contacts. For example, the duration (sec) of contacts was suppressed (by 72%) compared to control mice. Thus, these test conditions permit an evaluation of reduced social exploration, which is a fundamental behavioral outcome in the present mouse model of prenatal infection.

We are in the process of setting up the animal model of prenatal infection in Balb/c mice. Experiments include a determination of the dose of influenza virus to be used in pregnant dams. Our protocol is to anesthetize mice with 10mg/kg xylazine and 100 mg/kg ketamine (Shi et al., 2003), and then inoculate intranasally (i.n.) with 30, 60, 100, and 150 pfu of virus in 0.05 ml Hanks’ balanced salt solution without Mg$^{++}$ or Ca$^{++}$ (HBSS, Gibco Laboratories, Grand Island, NY). Sham or mock infection controls receive an i.n. inoculation with 0.05 ml HBSS containing no virus. A dose that results in
significant body weight loss without mortality is then used to infect a group of pregnant dams at 9.5 days to determine whether this dose is also sublethal in pregnant dams. Selecting an appropriate dose, we will begin a translational study to determine whether immunization against influenza virus before pregnancy prevents autistic-like behavior in the offspring of mothers infected during mid-pregnancy. Although vaccination is suggested before pregnancy, compliance is not universal, and it has not previously been tested for its potential ability to prevent behavioral disturbances associated with maternal infection. Adult female Balb/c mice will be infected with influenza virus. 1-month later, the mice will be paired with a breeder, and on day gestation day 9.5 will be divided into two groups: an infected group and a sham infected group. Mice will be allowed to come to term, and postnatal handling and testing will be conducted. We predict that behavioral disturbances associated with maternal infection will be prevented by vaccination.

**Projected Expenditures for Year 1.** The following Table itemizes the projected expenditures for Year 1 of funding.

**NJ Governor's Council on Autism**  
PI: Steven S. Zalcman, PhD  

<table>
<thead>
<tr>
<th>Category</th>
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<td>Supplies</td>
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<td>(Materials for immunological work, ELISA kits, glassware, plasticware, etc.)</td>
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PEER-REVIEWED PAPERS GENERATED BY THE 2007 COUNCIL-FUNDED BASIC SCIENCE AND CLINICAL RESEARCH GRANTS
Research Report

VPA-induced apoptosis and behavioral deficits in neonatal mice

Carrie L. Yochum, Peter Dowling, Kenneth R. Reuhl, George C. Wagner, Xue Ming

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ABSTRACT

Sodium valproate (VPA) administered to neonatal mice causes cognitive and motor deficits similar to those observed in humans with autism. In an effort to further evaluate similarities between early VPA exposure and autism, the present study examined treated mice for deficits in social behavioral and for neuronal damage. BALB/c mice injected on P14 with 400 mg/kg VPA engaged in fewer social interactions (including ano-genital sniffing, allogrooming, and crawling/overbehaviors) than control mice. Treated mice also exhibited reduced motor activity in a social context but were not significantly different from controls when motor activity was assessed in non-social settings. A second set of BALB/c mice were treated with VPA on P14 and sacrificed at different times thereafter for histopathological analysis. At time-points 12 and 24 h following VPA, treated mice had up to a 30-fold increase in the number of TUNEL-positive cells in the external granule cell layer of the cerebellum and a 10-fold increase in TUNEL-positive cells in the dentate gyrus of the hippocampus. These observations may provide a histopathological correlate for the social deficits observed following post-natal VPA exposure and supports the use of early VPA administration as an animal model for the study of autism.

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1. Introduction

Autism is a complex developmental disorder with core symptoms that include impaired social interactions, deficits in verbal and non-verbal communication, and the appearance of stereotypic and sometimes self-injurious behaviors. Post-mortem histological analysis of the brains of individuals with autism revealed a reduction in the number of cerebellar Purkinje cells (Bauman and Kemper, 1985, 1998, 2005; Ingram et al., 2000). Bauman and Kemper (1985) also found a decrease in cerebellar granule cell density in two of their case studies. In addition to these observations that cerebellar cell numbers are reduced, Paterni (2002) showed that the size of cerebellar Purkinje cells is also reduced. Taken together, these studies have been interpreted to indicate that permanent alterations in cerebellar Purkinje and granule cells may be neuroanatomical markers found in adult individuals with autism. However, in a study by Bailey et al. (1998), there appeared to be no localized pathology underlying autism, including no change in cerebellar Purkinje cell number or size. Given these studies these studies were post-mortem, they have several inherent limitations that may account for this discrepancy including a lack of controls for institutionalization, medication history, and co-morbid diagnosis of mental retardation. Studies using animal models of autism can control these types of variables and, perhaps, link anatomical and behavioral deficits. One important animal model is the study of...
model of autism involves early administration of sodium valproate (VPA) to rodents.

Clinical studies of children exposed to VPA in utero have characterized a fetal valproate syndrome with symptoms similar to autism, including deficits in language and communication, the appearance of stereotypic behavior, hyperexcitability and global delays in behavioral development (Ardinger et al., 1998; Koch et al., 1996; Mawer et al., 2002; Moore et al., 2000; Williams et al., 2001). Clinical case studies of infants with fetal valproate syndrome also report physical abnormalities including low myelomeningocele lesion, minor abnormalities of the face and ear, and microcephaly (Ardinger et al., 1998; Moore et al., 2002). These clinical similarities have led Rodier (1997) to propose that prenatal VPA exposure in rodents might serve as an animal model of autism.

Prenatal VPA treatment has been shown to affect behavioral development with treated rats exhibiting repetitive/stereotypic-like behavior, decreased exploratory activity, and a decreased number of social behaviors (Voorhees, 1986; Schneider and Przewotcki, 2004). Likewise, VPA exposure in rodents at the time of neural tube closure has been shown to reduce the number of cerebellar Purkinje cells (Ingram et al., 2000; Sobaniec-Lotowweska, 2001). These observations lend credibility to the suggestion that early exposure to VPA in rodents leads to behavioral and neurological deficits that resemble autism.

Previously, we demonstrated that both pre- and early postnatal exposure to VPA lead to neurodevelopmental deficits that are similar to the motor and cognitive deficits seen in humans diagnosed with autism (Wagner et al., 2006). In that study, the neurobehavioral deficits induced by early VPA exposure were classified as retardations (i.e., a behavioral skill that matured later in the treated mice as compared to controls), regressions (i.e., a behavioral skill that matured on schedule but was then lost subsequent to VPA exposure), or intrusions (i.e., the VPA exposure induced behaviors aberrant in intensity or frequency that overshadowed the normal maturation of behavioral skills). The behavioral tests assessed broad categories of motor and cognitive performance but, in this first study, the maturation of social skills following VPA exposure was not addressed. In addition, there was no assessment of the VPA-induced neural damage.

Accordingly, the objectives of the present study were to advance the VPA model of autism in mice by evaluating social behaviors and neuronal apoptosis following exposure on postnatal day 14 (P14). Wagner et al. (2006) demonstrated that critical cerebellar-mediated behaviors first appear on P14. Furthermore, a single 400 mg/kg postnatal exposure on P14 was effective in producing VPA-induced motor and cognitive deficits that mimicked autistic regression. Therefore, the current study used an acute dose of VPA at P14 in order to determine if a single exposure would also result in social deficits in the developing mice. The second objective was to determine if the P14 VPA treatment affected the integrity of the cerebellum and/or hippocampus, two regions associated with autism in humans. Postnatal day 14 roughly corresponds to the third trimester in human development (Rice and Barone, 2000), a period when hippocampal and cerebellar granule cells are undergoing migration and differentiation (Bachevalier and Beauregard, 1993; Rice and Barone, 2000; Voorhees, 1986). Accordingly, we examined these regions using the TUNEL stain for apoptosis.

2. Results

2.1. Social behavior

Social behavior is a primary deficit in autism. In order to monitor whether postnatal exposure to VPA would alter social behaviors in mice, treatment-matched pairs were observed for social and play behaviors in an open field environment. There was a significant effect of treatment for ano-genital sniffs [F(1,18) = 17.31, p = 0.001], crawl-under/over behaviors [F(1,18) = 5.42, p = 0.03], and allogrooming [F(1,18) = 7.16, p = 0.015] as shown in Fig. 1. Decreased ano-genital sniffing, crawl-under/over behavior and...
Fig. 2 – Number of horizontal movements made by sex- and treatment-matched pairs of BALB/c mice over a 30 min open field trial (run between post-natal days 30–40) following P14 sodium valproate (VPA, 400 mg/kg s.c.) or saline treatment (n = 12). Number of horizontal movements made by unpaired BALB/c mice in a 30 min open field trial (run between post-natal days 30–40) following sodium valproate (VPA, 400 mg/kg s.c.) or saline treatment in BALB/c pups (n = 12) for a total of 40 mice. *indicates significantly different from saline treated mice.

allo-grooming were observed in pairs of VPA-treated mice compared to controls. There was no significant difference for non-social behaviors such as self-grooming (data not shown).

2.2. Motor activity

Motor activity of the pair of mice was monitored during the social interaction and play test. There was a significant effect of treatment on motor activity levels (F(1,180) = 6.95, p = 0.017) such that VPA-treated pups engaged in fewer horizontal movements over the 30-min test period than control pups (as shown in Fig. 2). In order to determine if the decrease in motor activity in VPA-treated mice was the result of a global motor impairment, a second motor test was performed with an additional group of mice that was identically treated and tested individually under the same conditions. Each mouse had a horizontal movement analysis which consisted of a total activity count for a 30-min period that was separated into three 10-min bins (equal to the amount of movement time analyzed during play behavior). There was a significant effect of treatment for horizontal movements in a non-social setting (F(1,17) = 6.60, p = 0.02) such that treated animals engaged in significantly more horizontal movement than the control animals (as shown in Fig. 2). Therefore, VPA-treated BALB/c mice had a higher amount of motor activity.

Peak Time-Point Examples

Cerebellum

VPA 100X

Hippocampus

VPA 100X

PL

ML

GL

CA3

DG

CA1

Fig. 3 – Seven-micron thick sagittal slice of the third lobe of the cerebellum and the hippocampus (original magnification ×10) at the peak time-point for number of cells positively stained for apoptosis (between 12 and 24 h) following sodium valproate injection (VPA, 400 mg/kg s.c.) on P14 in BALB/c pups. GL indicates the granular cell layer of the cerebellum. PL indicates the Purkinje cell layer of the cerebellum. ML indicates the molecular layer of the cerebellum. DG indicates the dentate gyrus section of the hippocampal structure. CA3 indicates the CA3 section of pyramidal cells in the hippocampal structure. CA1 indicates the CA1 section of pyramidal cells in the hippocampal structure.

Please cite this article as: Yochum, C.L., et al., VPA-induced apoptosis and behavioral deficits in neonatal mice, Brain Res. (2008), doi:10.1016/j.brainres.2008.01.055
Cerebellar Regions
12 Hour Sacrifice Time-point  24 Hour Sacrifice Time-point

Fig. 4 – Photomicrographs of 7-μ thick sagittal sections of the granular cell layer of the third lobe of the cerebellum (original magnification ×40) at the time-points of 12 and 24 h following either sodium valproate injection (VPA, 400 mg/kg s.c.) or saline control on P14 in BALB/c pups.

Hippocampal Regions
12 Hour Sacrifice Time-point  24 Hour Sacrifice Time-point

Fig. 5 – Photomicrographs of 7-μ thick sagittal sections of the dentate gyrus section of the hippocampus (original magnification ×40) at the time-points of 12 and 24 h following either sodium valproate injection (VPA, 400 mg/kg s.c.) or saline control on P14 in BALB/c pups.
activity in a non-social environment and a lower amount of motor activity in a social environment as compared to saline controls. These results suggest that decreased activity in a social context was not the result of motor impairment.

2.3. TUNEL stain

BALB/c pups were injected on P14 (saline or VPA 400 mg/kg) and sacrificed 6, 12, 24, or 48 h later. Histochemical analysis of brain tissue was performed using the TUNEL stain for apoptosis (Fig. 3). Cerebellum and hippocampus showed substantially higher levels of TUNEL stain following treatment than other brain regions. Therefore, we focused on these regions in our analysis. The cerebellum and the hippocampus were analyzed using the Scion Image program (Figs. 4, 5).

![TUNEL stain graph]

**Fig. 6** - Average number of granule cells positively stained for apoptosis taken from three sections of 7-μ thick sagittal slices of the granule cell layer from cerebellar lobe three (original magnification ×40) at the time-points of 6, 12, 24, and 48 h following either sodium valproate injection (VPA, 400 mg/kg s.c.) or saline control on P14 in BALB/c pups (n = 6). Average number of granule cells positively stained for apoptosis taken from three randomly chosen sagittal sections of the dentate gyrus area of the hippocampus (original magnification ×40) at the time-points of 6, 12, 24, and 48 h following either sodium valproate injection (VPA, 400 mg/kg s.c.) or saline control at P14 in BALB/c pups (n = 6). *indicates significantly different from saline treated mice.

2.3.1. Cerebellum

There was a significant effect of treatment \(F(1,40) = 52.48, p < 0.001\) and sacrifice time point \(F(3,40) = 17.28, p < 0.001\) on the number of TUNEL-positive cells in the cerebellum. There was also a significant treatment by time point interaction \(F(3,40) = 16.25, p < 0.001\) in the cerebellum (as seen in Fig. 6). The number of TUNEL-positive cells occurring in the 177 VPA-treated animals was significantly increased at the 12 and 24 h time point in the cerebellum. Post hoc test shows that the 179 VPA-treated 12 h time point animals have a significantly higher TUNEL-positive cell count than the 24 h.

2.3.2. Hippocampus

There was a significant effect of treatment \(F(1,40) = 40.99, p < 0.001\) and sacrifice time point \(F(3,40) = 9.33, p < 0.001\) on the 184 hippocampus. There was also a significant treatment by time point interaction \(F(3,40) = 9.89, p < 0.001\) on the number of 186 TUNEL-positive cells in the hippocampus (as seen in Fig. 6). The 187 number of TUNEL-positive cells occurring in VPA-treated animals 188 was significantly increased at the 12 and 24 h time-points in the 189 hippocampus. Post hoc analysis revealed that the VPA-treated 190 animals sacrificed at the 24 h time point have a significantly 191 higher number of TUNEL-positive cells than the 12 h time point.

3. Discussion

In order to determine whether post-natal VPA affects social behavior, VPA-treated and control BALB/c mouse pups were scored for social and non-social behaviors over a 30 min period. Treated pups performed significantly fewer social behaviors than control animals. In addition, when treated animals were placed in a non-social environment, their activity level was higher than that of control animals. Therefore, the VPA-induced decrease in social behavior does not reflect a motor deficit but was specific to the social context. The observation that the P14 202 VPA treatment affects social behavior supports the contention that early exposure to VPA provides a useful animal model of autism (Rodier et al., 1996; Wagner et al., 2006; Tsuji, et al., 2007; Markram et al., 2007). In our previous study, the acute effects of VPA were thoroughly examined and it was observed that there was a slight and transient reduction in body weight 209 (Wagner et al., 2006). In the present study, we observed a short period of lethargy and a slight transient reduction in body weight with essentially no increased mortality for VPA-treated pups. These changes were short term and pups had fully recovered by the third day following treatment, which was 214 weeks before their first day of social testing. This indicates that the social behavioral deficits observed in this study may be classified as a behavioral "retardation" and not the result of an acute pharmacological effect. Thus our present findings advance the model previously proposed by Wagner et al. (2006) with the observation that early VPA exposure causes deficits in social behavior, a core symptom of autism.

We also demonstrated that VPA treatment enhanced cell death in the cerebellum and hippocampus. The cerebellum of treated mice had up to a 30-fold increase in TUNEL-positive cells over the control baseline at the 12 h sacrifice time point. In addition, the hippocampus had up to a 10-fold increase in TUNEL-positive cells over baseline at the 24 h sacrifice time point.
4. Experimental procedures

4.1. Animals

Adult male BAL/c mice (Jackson Laboratory, Bar Harbor) were introduced into a cage of two adult females; females were checked every morning thereafter for the presence of vaginal plug (embryonic day zero). Pregnant mice were then removed and housed singly. All animals were maintained under standard housing conditions, with free access to food and water and a 12:12 h light/dark cycle, in accordance with AAALAC guidelines.

4.2. Groups design

Mice were injected subcutaneously with either saline or VPA 400 mg/kg (Sigma) on postnatal day 14 of life. A total of 20 pairs (40 mice; 12 saline pairs and 8 VPA pairs) were assessed for behavioral tasks that comprised the social interaction and play study on P30–P40. We used a total of 13 different litters. Within each pair, pups were matched for age, sex, and treatment, with no pair of pups drawn from the same litter. Furthermore, within each individual litter, the pups were randomly assigned to either the VPA or saline treatment.

The apoptosis study tested a separate group of mice that totaled 48, with 12 animals at each time point (6 saline treated and six VPA-treated mice at each time point). Mice in this study were injected subcutaneously with either saline or VPA 400 mg/kg (Sigma) at a concentration of 40 mg/ml on postnatal day 14 of life and sacrificed 6, 12, 24 or 48 h thereafter.

4.3. Social interaction and play

The critical time period during which pup social behavior (play behavior) first appears ranges from postnatal day 30 to day 40 (Ricciari et al., 2000). Naive mice between P30–40 were individually housed 4–5 days prior to the test session. Pairs of non-sibling BAL/c mice were then observed for social interactions for one 30 min session and scored by two trained observers for the number of times that a member of the pair engaged in a behavior. All observers were trained by the first author, on mouse pairs not used in this study, until there was a 98% agreement in the observer scores. These observers then scored pairs of animals blind to treatment condition. Testing was conducted at the start of the dark cycle and the testing room was illuminated by red light only. During testing, the behaviors they observed were: ano-genital sniffs, face sniffs, crawl-under/over behaviors, self-grooming, and allogrooming (allogroom behaviors were defined as one mouse rising up on its hind legs to touch paws and snout to the other mouse to perform grooming motions).

4.4. TUNEL assay

The apoptosis identification technique used was the ApopTag kit which uses a TUNEL assay (Chemicon manual). Pups were sacrificed 6, 12, 24 or 48 h following the P14 injection and whole brains were placed in 10% neutral buffered formalin for 24 h, rinsed in PBS, and stored in a 70% ethanol solution until the day of paraffin embedding. One VPA brain and one saline brain of 338 the same sex and sacrifice time point were embedded side by side...
4.5. **Cell counting**

Nine sections were taken from each brain starting from midline outward (brains were embedded parasagittally). Photomicrographs were taken at 400x for each of these brain slices. All cerebellar photomicrographs were taken from the external granule cell layer of anterior lobe three of the cerebellum in order to standardize sections used for cell counts. All hippocampal photomicrographs were taken from the dentate gyrus. These similar regions were then compared using the Scion Image program with particle size and density index set at a constant. Particle size and density settings were determined by matching Scion counts with manual counting under microscope.

4.6. **Statistical analysis**

All data were analyzed using SPSS statistics package. A repeated measures ANOVA including both group and sex as main factors were used for all behavioral tests. For TUNEL cell counting, multifactorial ANOVA including treatment, timepoint and sex as main factors was used with Tukey’s HSD as a posthoc test. P values <0.05 were considered statistically significant. There was no sex effect observed on any of our analyses.

5. **Uncited reference**

Rodier et al., 1997

R E F E R E N C E S


Please cite this article as: Yochum, C.L., et al., VPA-induced apoptosis and behavioral deficits in neonatal mice, Brain Res. (2008), doi:10.1016/j.brainres.2008.01.055
Evidence of Oxidative Stress in Autism Derived from Animal Models

Xue Ming, Michelle A. Cheh and Carrie L. Yochum, Alycia K. Halladay and George C. Wagner

Pediatric Neuroscience, UMDNJ, Newark, NJ
Psychology, Rutgers University, New Brunswick, NJ
Autism Speaks, Princeton, NJ

Abstract: Autism is a pervasive neurodevelopmental disorder that leads to deficits in social interaction, communication and restricted, repetitive motor movements. Autism is a highly heritable disorder, however, there is mounting evidence to suggest that toxicant-induced oxidative stress may play a role. The focus of this article will be to review our animal model of autism and discuss our evidence that oxidative stress may be a common underlying mechanism of neurodevelopmental damage. We have shown that mice exposed to either methylmercury (MeHg) or valproic acid (VPA) in early postnatal life display aberrant social, cognitive and motor behavior. Interestingly, early exposure to both compounds has been clinically implicated in the development of autism. We recently found that trolox, a water-soluble vitamin E derivative, is capable of attenuating a number of neurobehavioral alterations observed in mice postnatally exposed to MeHg. In addition, a number of other investigators have shown that oxidative stress plays a role in neural injury following MeHg exposure both in vitro and in vivo. New data presented here will show that VPA-induced neurobehavioral deficits are attenuated by vitamin E as well and that the level of glial fibrillary acidic protein (GFAP), a marker of astrocytic neural injury, is altered following VPA exposure. Collectively, these data indicate that vitamin E and its derivative are capable of protecting against neurobehavioral deficits induced by both MeHg and VPA. This antioxidant protection suggests that oxidative stress may be a common mechanism of injury leading to aberrant behavior in both our animal model as well as in the human disease state.

Key words: Vitamin E, trolox, valproic acid

INTRODUCTION

The core symptoms of autism include language deficits, impaired social interactions and inappropriate, stereotypic and sometimes self-injurious behaviors. The etiology of autism remains unknown but may involve early exposure to environmental toxicants acting upon genetically-sensitive individuals. No single toxicant has been identified; rather a broad range of toxicants including drugs, metals, solvents, herbicides, pesticides, etc. have been associated with autism. A common feature across this range of potential compounds is toxicant-induced oxidative stress causing neuronal damage leading to the behavioral phenotype of autism. Likewise, no single gene has been identified but, rather, a constellation of as many as 15 polymorphisms may ultimately predispose the individual to autism. Again, genetic alterations leading to compromised handling of toxicant-induced reactive oxygen species (ROS) has been a common theme.

Since the etiology of autism is unknown, it is essential that animal models be developed. The behavioral symptoms of autism have proven difficult to model in other species. Accordingly, we have initiated work on a novel strategy to model the behavioral phenotype of autism in mice. In this model, the normal development of key behaviors is carefully monitored from birth through adolescence. Once the maturation of these key behaviors is understood in terms of the postnatal day(s) of life in which subjects are able to successfully perform the task or engage in the behavior, the performance of mice with early toxicant exposure and/or genetic modification can be assessed.

The model strategy begins by characterizing behavioral manifestations of developmental disorders as retardations (a behavior fails to develop during a critical period of maturation), regressions (a behavior develops at about the right time but then is lost with

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later development, especially following toxicant exposure, or intrusions (the appearance of behaviors aberrant in form or frequency which mask normal development). Most developmental disorders include some combination of these conditions. In this framework, the hypothesis that environmental toxicants or genetic alterations are causally involved in autism can be readily tested. That is, acute or repeated exposure to a toxicant should disrupt neurobehavioral development causing behavioral retardation, regression, or intrusions and these toxicant-induced behavioral deficits should occur at lower doses in the genetically-sensitive mice. Traditionally, animal models of developmental disorders have not examined these three scenarios of retardations, regressions and/or intrusions but, instead, focus on single aspects of neurobehavioral development. The judicious use of toxicants associated with autism or toxicants known to damage brain regions associated with autism confers some selectivity of the model for autism. Likewise, manipulation of genes associated with autism also confers some selectivity of this model for autism. Finally, administering a battery of tests that assess social, cognitive and motor maturation of the mice confers some selectivity for autism. Ultimately, it is the possibility of combining select toxicant exposure in genetically-sensitive mice followed by thorough assessment of social, cognitive and motor skill maturation that makes this a comprehensive animal model of autism.

In our initial studies, we identified toxicant induced retardation of motor and cognitive skills following prenatal exposure to sodium valproate (VPA). Likewise, we were able to demonstrate dramatic loss of acquired skills, i.e. regressions, following postnatal VPA administration. Finally, we demonstrated toxicant induced intrusions wherein toxicant-treated subjects exhibited dramatic increases in stereotypic and self-injurious behaviors akin to those seen in autism.

VPA was chosen as our first agent to test this model following reports of an association between autism and prenatal exposure to this teratogen. Previous studies have also demonstrated impairment in cognitive, motor, attention and social development in rats administered pre- or post-natal VPA. Accordingly, in our first studies mice were exposed to VPA either in utero or post-natally. The prenatal exposure time reflected a period of cerebellar Purkinje cell generation differentiation in the mouse. The post-natal time of P14 was based on our observation that critical cerebellar-mediated behaviors of mid-air righting and negative geotaxis mature or first appear on this day in the mouse and because of continued neuronal and glial development in other brain regions. Of importance, VPA administration results in high levels of markers for oxidative stress and lipid peroxidation including 15-F-iso prostane and thiobarbituric acid reactive substances.

An organic mercury, MeHg, was selected as our second compound for testing because it is an important, widely distributed environmental toxicant. MeHg does cross the placental barrier and, in humans exposed in utero to acute high doses, was shown to cause retardation in cognitive and locomotor development along with numerous other neurological symptoms including seizures and cerebral palsy. Nonetheless, it is important to note that autism was not found to be associated with either pre- or neonatal exposure to organic mercury.

The consequences of low dose, chronic exposure to mercury through fish consumption are somewhat more controversial with some studies showing deleterious effects while others show no adverse consequences.

Early exposure to mercury has been shown to disrupt the neurodevelopmental development of other species including rodents and primates. The mechanism through which MeHg exerts its toxicity is thought to be, in part, mediated by disruption of neural cell adhesion molecules. In addition, oxidative stress is involved in MeHg-induced neurotoxicity as demonstrated by increased ROS and thiobarbituric acid reactive substances and a reduction in GSH levels. In addition, the neurotoxicity of MeHg in cultured neurons was blocked by the pretreatment with antioxidants. Trolox, a water-soluble derivative of vitamin E, protects against MeHg-induced neurotoxicity in rats. Likewise, antioxidants produced protective effects against MeHg toxicity in cultured human neurons and astrocytes. Indeed, ROS have been implicated in MeHg-induced neurotoxicity in multiple experimental models. Finally, we have recently demonstrated thatpretreatment with Trolox protects mice against the neurobehavioral deficits induced by postnatal MeHg. Collectively, these data indicate that early exposure to MeHg causes neurobehavioral deficits consequent, at least in part, to the generation of ROS.

In summary, wide ranges of toxicants and genetic alterations have been associated with autism. The toxicants are thought to have a common mechanism of generating ROS while the genetic alterations are thought to result in enhanced sensitivity to the deleterious effects of ROS. Accordingly, we now hypothesize that autism may be the result multiple exposures to any of a number of toxicants; the initial exposure sensitizes the subject such that later exposures to the same or different toxicants results in an enhanced
oxidative stress response. Furthermore, we predict that this sensitization will be exacerbated in individuals with genetic alterations affecting their handling of ROS. In previous studies we have demonstrated a sensitization response to dopaminergic toxicants in adult mice following prenatal administration of MeHg\(^\text{[7]}\). We have also demonstrated that antioxidant pretreatment protects mice against the behavioral deficits induced by early exposure to MeHg\(^\text{[8]}\). Accordingly, the objective of this study was to determine if antioxidants administered as a pretreatment to VPA would protect the mice against the VPA-induced behavioral regression. In addition, we sought to determine if the early VPA administration would alter levels of glial fibrillary acidic protein (GFAP), a marker of astrocytic neural injury, thus serving as a biological marker for the VPA-induced behavioral deficits.

**MATERIALS AND METHODS**

**Subjects:** Male and female BALB/c mice (Taconic, Germantown, NY) were housed together in plastic cages with standard wood chip bedding and free access to food and water. All mice were maintained in an AAALAC-accredited facility under guidelines set forth by the National Institutes of Health. Lights were set on a 12 h on: 12 h off cycle and temperature was maintained at 25°C. Females were checked before 10 AM for presence of a vaginal plug which was recorded as day 0 of embryonic development. Day of birth was recorded as day 0 and all pups were labeled for individual identification. Body weight was measured daily. Female pups were removed from the cage on day 5. For the behavioral studies, the sodium valproate (Sigma) dose was 400 mg kg\(^{-1}\) with a saline vehicle and the vitamin E dose was also 400 mg kg\(^{-1}\) but with a corn oil vehicle. All injections were s.c. in a volume of 1.0 mL 100\(^{-1}\) g body weight.

**Negative geotaxis:** Negative geotropism was tested on postnatal days P13-19 by placing the mouse facing downward along a 45°C incline. Latency to turn 180°C such that the head was facing upward along the incline was recorded with a maximum of 30 seconds for each trial.

**Motor Activity:** Motor activity was assessed on days P14-19. The chamber consisted of a black 42x22x14 cm Plexiglass box. Six infrared sensors placed approximately 7 cm apart and 2.5 cm above the floor were used to measure activity over a 10 min period.

**Mid-air righting:** When a mouse is dropped upside down from a height of 45 cm onto a padded surface it engages in a mid-air righting reflex with orderly, rostro-caudal movements, initiated with head and concluded with the hindlimbs such that the animal lands on its paws. The behavior first appears on P13 and is fully achieved by P17\(^\text{[5]}\). Mid-air righting has been linked to cerebellum development\(^\text{[36]}\). For the mid-air righting test, mice were elevated 45 cm above a foam pad, dorsal side down. The animal was released and ability to right in mid air assessed scored as the mouse landing on its paws on two out of three trials each day. Mice were tested on P13-20.

**Protein determination:** In order to determine changes in protein expression following VPA treatment at behaviorally significant time points, animals were treated with VPA 600 mg kg\(^{-1}\) or saline on E13\(^\text{[1]}\) and assayed on days P4 and P5 with the cerebellum removed and stored at -70°C. Protein analysis via gel electrophoresis, western blot and densitometry was performed according to the methods of Dey et al.\(^\text{[56]}\) with some small variations. In summary, whole homogenate fractions were homogenized in 1:10 w/v of a Tris extraction buffer [50 mM TrisHCl, pH 7.4, 0.32M sucrose, 1 mM EDTA, 1 vial to 100 ml protease inhibitor (Sigma, St. Louis, MO)]. The supernatant was removed following centrifugation for 10 min at 1000xG and combined with equilibration buffer [0.125M TrisHCl, pH 6.8, 4% SDS, 20% glycerol, 10% mercaptoethanol] and immediately heated for 30 min at 70°C. Protein values were determined using the BCA protein assay (Pierce, Rockford, IL) modified for a BOBAS FARA II enzyme analyzer (Roche Diagnostics, Nutley, NJ). Samples of 10 μg protein were separated by SDS-PAGE on a 10% polyacrylamide gradient gel using a Bio-Rad Mini-Protean II System (Bio-Rad, Hercules, CA) for GFAP and synaptophysin. Proteins were transferred to nitrocellulose membranes and were washed twice for 10 min each in phosphate buffered saline (PBS) and blocked with 5% non-fat dry milk in PBS for 1 h prior to application of primary antibody. Immunoblotting for GFAP and synaptophysin was performed overnight at 4°C. All primary antibodies were obtained from Fisher (Springfield, NJ). Antigens were visualized following 1 h incubation with secondary peroxidase antibodies (Southern Biotechnology Associates, Birmingham, AL) and application of chemiluminescence ECL substrate detection on Hyperfilm ECL autoradiographic film (Amersham). For GFAP detection, this method was verified in a separate study using a dose response of trimethyltin treatment using a GFAP protein standard.
(Chemicon, Inc.). ECL images were scanned into an IBM PC using a Hewlett Packard Scanner with a transparency adapter. Densitometric analysis was performed using Image Pro Analysis Software using percent of saline treated controls as the standard.

**Statistical analysis:** All behavioral analysis were performed using a repeated measures ANOVA including both group, day and sex as main factors, with the exception of the mid-air righting response which was analyzed using Chi-Square and Fisher’s Exact Test.

**RESULTS AND DISCUSSION**

**Negative geotaxis:** Control mice and those treated with vitamin E alone were able to perform the reflexive negative geotaxis response, reorienting their head to point upward when placed on an inclined plane with their head facing down. This reflex improved across development, as the latency to re-orient improved across testing (F(6, 312) = 5.4, p<0.001). VPA-treated mice displayed an increased latency to perform this re-orientation response (F(1, 52) = 10.0, p<0.005). Post-hoc analysis revealed that following day 14 treatment with VPA, there was a significant regression in the performance of this response, which reached statistical significance on days 16 and 17. Importantly, this VPA-induced regression was blocked by vitamin E pretreatment, such that pretreated mice were able to perform this response with a similar latency as controls on P16 and P17 (F(1, 52) = 5.3, p<0.05). Finally, VPA-treated mice regained their ability to perform this behavioral response similar to controls by the completion of testing on P19 (Fig. 1).

**Mid-air righting:** Number of pups successfully engaging in mid-air righting (expressed as a percent of pups mid-air righting on 2 out of 3 trials/day) for groups of pups treated with VPA (400 mg kg⁻¹) or saline on P14. Some groups received vitamin E pretreatment while others received corn oil. *: p<0.05 compared to corn oil/saline

**Motor activity:** Mice engaged in a similar amount of locomotor activity at the start of testing. This activity significantly increased across post-natal development [F(1, 38) = 80.2, p<0.001]. Interestingly, there was a trend for mice treated with VPA to engage in intrusive behaviors following P14 treatment. This was evidenced by increased levels of activity across testing, beginning on P16 through P18. However, this hyperactivity did not reach statistical significance. In addition, this hyperactive behavior was blocked by pre-treatment with vitamin E (Fig. 3).

**GFAP and synaptophysin:** Previous work in our lab revealed retarded neurobehavioral development in mice treated with 600 mg kg⁻¹ VPA on embryonic day 13[1]. In order to determine whether we could detect a
Fig. 3: Motor activity: Horizontal beam breaks for groups of pups treated with VPA (400 mg kg\(^{-1}\)) or saline on P14. Some groups received vitamin E pretreatment while others received corn oil. *: p<0.05 compared to corn oil/saline.

Fig. 4: GFAP: Pups received either VPA (600 mg kg\(^{-1}\)) or saline \textit{in utero} on E13 and were sacrificed on either P4 or P5. *: p<0.05 compared to corn oil/saline.

Fig. 5: Synaptophysin: Pups received either VPA (600 mg kg\(^{-1}\)) or saline \textit{in utero} on E13 and were sacrificed on either P4 or P5. *: p<0.05 compared to corn oil/saline.

(F (2, 6) = 10.0, p<0.01) (Fig. 5). During this period of postnatal development, GFAP and synaptophysin levels increase to promote normal astrocytes-neuron interactions and synaptogenesis, respectively\(^{[37]}\). Therefore, mice treated with VPA \textit{in utero} show immature neural development, since the levels of GFAP and synaptophysin observed on P5 are much lower than those found in mice from an earlier postnatal period (P4). This suggests that the behavioral retardations seen in our previous study are influenced by retarded neural development.

The etiology of autism is thought to involve early exposure to ROS-generating toxins acting upon genetically-sensitive individuals. We have developed a new strategy to assess the detrimental effects of early toxicant exposure on neurobehavioral development, classifying the behavioral deficits as retardations, regressions or intrusions. In previous studies we demonstrated that early exposure to VPA or MeHg results in behavioral deficits in the maturation of social, cognitive and motor skills\(^{[8]}\). Furthermore, we demonstrated that our behavioral model was useful in demonstrating that pretreatment with an antioxidant protected mice against the behavioral deficits induced by early exposure to MeHg\(^{[8]}\). In the present study, we demonstrated that vitamin E was capable of protecting mice against VPA-induced regression in negative geotaxis and mid-air righting as well as against intrusive VPA-induced hyperactivity. Collectively, the present data together with our previous MeHg study indicate that the generation of ROS may be a common
factor mediating toxicant-induced neuronal damage associated with autism and that neurobehavioral assessments provide an important functional measure of the potential benefits of antioxidants.

A second objective of the present study was to develop a biological marker of the VPA-induced damage. Toward this end we used our initial model, delivering the VPA prenatally on E13. We had demonstrated that this prenatal VPA treatment resulted in later behavioral deficits as assessed in the surface and mid-air righting tests, negative geotaxis and in water maze. Furthermore, this prenatal VPA treatment resulted in sex-dependent differences in these behavioral deficits with males more affected than females. In the present study, we found that both cerebellar GFAP and cerebellar synaptophysin were reduced postnatally following the prenatal VPA administration. GFAP is a marker of astroglia in the brain and is involved in astrocyte-neuron interactions. GFAP mutant mice have abnormal structure and exhibit deficient long-term depression in cerebellar Purkinje cell synapses. Therefore, major alterations in GFAP may alter Purkinje cell communication that, in turn, may alter behavior. Synaptophysin is a widely used marker for nerve terminals and can indicate synaptogenesis. Therefore, a reduction in synaptophysin in the cerebellum could signify a reduction in synaptogenesis in that region. More generally, it is intriguing that these biological markers may reflect the behavioral deficits of cognitive and motor retardation caused by the early VPA exposure. Future studies are designed to determine if the antioxidant pretreatment also protects the mice against these neurological changes induced by the VPA.

There is ample evidence that ROS are involved in human autism. Free oxygen radicals could result from ingested or inhaled environmental toxins, food or food additives, inflammation or infection (overt or occult). The interaction of free oxygen radicals and polymorphic oxidative genes during gestation or postnatally could disrupt neurogenesis in developing brain at multiple time windows, eliciting immediate stage-dependent effects in specific systems that influence subsequent ontogenetic processes, leading to the phenotype of autism. Indeed an exacerbated oxidative stress response has been implicated in autism. Specifically lower glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity were found in children with autism. An increase in body burden of various toxins was reported in autism. In addition, provoked urinary mercury excretion is found to be higher in autism. These toxins could generate oxidative stress in children with autism. Elevated nitrite and nitrate in plasma and red cells have been reported in children with autism. This elevation indicates excess generation of nitric oxide free radicals. In addition, two independent double blind placebo controlled clinical trials of antioxidants (vitamin C or carnosine) showed beneficial effects in autism. Finally, we conducted a study of oxidative stress biomarkers in children with autism and age matched healthy controls. Our results showed that urinary excretion of 8 isoprostane F2α was significantly higher in children with autism as compared to healthy controls. There was also a trend of increased 8-OHDG urinary excretion in autistic subjects. These results suggest that oxidative stress is exacerbated in autism and are consistent with the present results of antioxidant protective effects against VPA-induced behavioral deficits in mice.

In summary, we have developed a comprehensive neurobehavioral model in which mice are exposed to candidate toxicants during critical periods of neural development. The mice may have altered expression of genes thought to be associated with autism and/or confer increased sensitivity to the toxicants. The mice are then assessed in a battery of tests designed to assess behavioral maturation of skills in the social, cognitive and motor domains. Toxicant or genetic-induced deficits in the behavioral maturation are classified as retardations, regressions or intrusions. In the present studies, we further demonstrate that pretreatment with an antioxidant protects the mice against the toxicant-induced behavioral deficits. We conclude that our model is useful for evaluation of the theory that oxidative stress may play a role in the etiology of autism.

ACKNOWLEDGEMENT

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REFERENCES


Original Contribution

Advanced Parental Age and the Risk of Autism Spectrum Disorder

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This study evaluated independent effects of maternal and paternal age on risk of autism spectrum disorder. A case-cohort design was implemented using data from 10 US study sites participating in the Centers for Disease Control and Prevention’s Autism and Developmental Disabilities Monitoring Network. The 1994 birth cohort included 253,347 study-site births with complete parental age information. Cases included 1,251 children aged 8 years with complete parental age information from the same birth cohort and identified as having an autism spectrum disorder based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria. After adjustment for the other parent’s age, birth order, maternal education, and other covariates, both maternal and paternal age were independently associated with autism (adjusted odds ratio for maternal age ≥35 vs. 25–29 years = 1.3, 95% confidence interval: 1.1, 1.6; adjusted odds ratio for paternal age ≥40 years vs. 25–29 years = 1.4, 95% confidence interval: 1.1, 1.8). Firstborn offspring of 2 older parents were 3 times more likely to develop autism than were third- or later-born offspring of mothers aged 20–34 years and fathers aged <40 years (odds ratio = 3.1, 95% confidence interval: 2.0, 4.7). The increase in autism risk with both maternal and paternal age has potential implications for public health planning and investigations of autism etiology.

autistic disorder; birth order; maternal age; paternal age

Abbreviations: ASD, autism spectrum disorder; PDD-NOS, pervasive developmental disorders— not otherwise specified.

This paper examines the relation between parental age at delivery and the prevalence of autism spectrum disorder (ASD). The possibility that autism is more common in offspring of older parents has generated considerable interest (1–6). Confirmation of such an association could have important public health implications in light of increasing trends in recent decades regarding both maternal and paternal age (7). In addition, evidence of paternal and maternal age effects on autism risk may provide clues to the etiology of a class of neurodevelopmental disorder that is still poorly understood and thought to be complex and multifactoral.

In evaluating the association between parental age and autism risk, it is important to account for other variables related to both parental age and autism or that may modify the association. Birth order is a potentially confounding factor because it is positively associated with parental age and has been reported in some studies to be associated with autism risk, with at least 3 studies reporting firstborn children to be at increased risk of autism (1, 2, 4). The goal of this study was to determine, in a large, population-based cohort of US children, whether advancing maternal and paternal age each independently increase a child’s risk of developing autism after controlling for the other parent’s age, birth order, and other risk factors.

MATERIALS AND METHODS

Study design and sample

We implemented a population-based, case-cohort design in which the comparison group was a cohort of all livebirths in 1994 in 10 geographically defined study areas participating in the Centers for Disease Control and Prevention’s Autism and Developmental Disabilities Monitoring Network (8). The
Table 1. Characteristics of the 1994 Birth Cohort and ASD Cases, 10 Study Sites From the US Centers for Disease Control and Prevention’s Autism and Developmental Disabilities Monitoring Network

<table>
<thead>
<tr>
<th></th>
<th>Full Birth Cohort: All Livebirths in the 10 Study Areas</th>
<th>Birth Cohort With Complete Information on Parental Age</th>
<th>All Children Aged 8 Years With ASD Residing in the 10 Study Areas in 2002</th>
<th>ASD Cases With Any Birth Certificate Information</th>
<th>ASD Cases With Complete Information on Parental Age</th>
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<tr>
<td>No. (%)</td>
<td>326,785 (100)</td>
<td>253,347 (77.5)</td>
<td>2,142 (100)</td>
<td>1,517 (70.8)</td>
<td>1,251 (58.4)</td>
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<tr>
<td>Median maternal age, years</td>
<td>27</td>
<td>28</td>
<td>29</td>
<td>29</td>
<td>29</td>
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<td>Median paternal age, years</td>
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<tr>
<td>Maternal age &lt;20 years, %</td>
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<td>8.5</td>
<td>8.0</td>
<td>5.4</td>
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<tr>
<td>Maternal age ≥35 years, %</td>
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<td>Boys, %</td>
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<td>51.4</td>
<td>81.2</td>
<td>81.7</td>
<td>81.8</td>
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<tr>
<td>White, %</td>
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<td>63.1</td>
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<td>Black, %</td>
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<td>16.5</td>
<td>22.1</td>
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<td>Hispanic, %</td>
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<td>Gestational age &lt;37 weeks, %</td>
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<td>13.6</td>
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<td>Gestational age &lt;28 weeks, %</td>
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<td>0.6</td>
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<td>1.5</td>
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<tr>
<td>Autistic disorder, %</td>
<td></td>
<td></td>
<td>81.0</td>
<td>80.8</td>
<td>80.7</td>
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<tr>
<td>Confirmed intellectual impairment, %</td>
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<td></td>
<td>32.4</td>
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<td>30.9</td>
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<td>Confirmed normal intelligence, %</td>
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<td></td>
<td>43.2</td>
<td>42.1</td>
<td>43.2</td>
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</tbody>
</table>

Abbreviation: ASD, autism spectrum disorder.
* Information to confirm intellectual functioning was missing for approximately 25% of ASD cases.

10 areas are all sites with deidentified birth certificate information on parental age and other relevant variables included in the Network database and include sites in Alabama, Arizona, Arkansas, Colorado, Georgia, Maryland, Missouri, New Jersey, North Carolina, and Wisconsin.

The cohort serving as the comparison group includes all livebirths to mothers residing in any 1 of the study areas in 1994, with complete information available from birth certificates on maternal and paternal age, birth order, and other variables. We used 2 data sources to construct the cohort: 1994 deidentified birth records for the Wisconsin study area provided by the Wisconsin Department of Health and Family Services and, for the remaining sites, the National Center for Health Statistics public use natality data files (9). The public use file includes county of residence for births in densely populated counties, which enabled us to ascertain deidentifed birth information for all births in most of the counties. We were unable to precisely obtain counts of births occurring in sparsely populated counties in which 13,043 (4.1%) of the study-area births occurred in 1994. For these counties, we obtained county-level aggregate information on the total number of births in 1994 and their distribution by variables such as maternal marital status, ethnicity, and age and selected a stratified random sample of deidentified birth records (equal in number and similar in distribution by maternal marital status, ethnicity, and age to all livebirths occurring in the respective counties in 1994) from sparsely populated counties of the state in which the study area was located. The full cohort included 326,785 livebirths, of which 73,438 (22.5%) were excluded because of missing paternal age. The cohort serving as the comparison group thus included the 253,347 livebirths with complete information on parental age and other key variables (Table 1).

The total number of children aged 8 years residing in the study areas in 2002 determined by the Autism and Developmental Disabilities Monitoring Network surveillance system to have an ASD was 2,142. Birth certificate information was available for 1,517 (70.8%) of these children, who were born in the same state as their state of residence in 2002. The remaining 29.2% of cases were excluded from this analysis because of missing birth certificate information. The case group for the present analysis was further restricted to the 1,251 children (58.4% of the total ASD case group) for whom information on both parents’ age as well as birth order and gestational age was available. Our final sample was comparable to the total population of ASD cases regarding demographic factors and ASD case characteristics (Table 1).

Case definition

ASDs include behaviorally defined neurodevelopmental disorders diagnosed through clinical observation, and they encompass impairments in social, communicative, and behavioral development, often accompanied by abnormalities in intellectual functioning, learning, attention, and sensory processing. For this study, children with ASD included members of the birth cohort residing in the study area in 2002 who met Diagnostic and Statistical Manual of Mental
Disorders}, Fourth Edition, Text Revision criteria for autistic disorder; pervasive developmental disorders not otherwise specified (PDD-NOS (http://www.cdc.gov/ncbddd/autism/overview_diagnostic_criteria.htm), including atypical autism); or Asperger's disorder (10) based on a comprehensive review of educational and clinical records by trained clinicians. Children were classified by clinician reviewers as having an ASD if they had either a documented previous classification of ASD (65%) or an evaluation record from an educational or medical setting indicating unusual behaviors consistent with ASD (35%). For children previously identified as having an ASD, case status was confirmed on the basis of evaluation records. For children without a documented ASD classification, data were abstracted on all relevant ASD and developmental behaviors from education or medical evaluations to determine whether behaviors described in the evaluations by clinical reviewers were consistent with the diagnostic criteria. Because case status was determined solely on the basis of information contained in evaluation records, and because Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria are less well defined for PDD-NOS than for autistic disorder, the surveillance protocol for determining whether a child could be classified as having PDD-NOS required documentation of at least 1 behavior considered to be an ASD discriminator, such as being oblivious to others when there is a clear social opportunity or demonstrating atypical and persistent focus on sensory input (11).

Of the 1,251 ASD cases, 80.7% were determined to meet criteria for autistic disorder, while there was insufficient information for those remaining to distinguish between autistic disorder, Asperger's disorder, or PDD-NOS. Information from standardized intelligence tests was available for approximately 75% of the ASD cases. On the basis of this information, children with ASD were classified as having intellectual impairment (an IQ of <70) versus normal intelligence. Further details regarding the 2002 Autism and Developmental Disabilities Monitoring Network sample and methodology have been reported previously (8, 11).

Analytic strategy and statistical methods

Potential for confounding effects of birth order, gender, and other variables was evaluated by first examining unadjusted associations between each potential confounder and the independent variables of maternal and paternal age as well as the dependent variable, ASD case status. Variables were considered to be potentially confounding factors if they were associated with both parental age and ASD. Unadjusted odds ratios with confidence intervals were computed to evaluate the magnitude of these associations, and unconditional logistic regression models were fit to estimate adjusted odds ratios and 95% confidence intervals. Statistical significance was evaluated by using chi-square tests for categorical variables and analysis of variance for continuous variables.

To enhance the comparability of our findings with those from other studies, we fit 2 types of models, 1 with parental ages categorized into 6 categories: <20, 20–24, 25–29, 30–34, 35–39, ≥40 years; and the other with parental age as a continuous variable with the odds ratio scaled to reflect a 10-year difference in age (4). Although we found the association between parental age and autism risk to be similar across the 10 sites, to adjust for potential site-to-site variability we included site dummy variables in all multivariable models. To evaluate interaction or modifying effects of each covariate and of ASD subtypes on the associations between parental age and ASD, we performed stratified analyses. We also tested interaction terms for maternal age by paternal age and 2-way and 3-way interaction terms for each parent's age by the other covariates in the regression models, but we identified no significant interactions. SAS version 9.1.3 software (SAS Institute, Inc., Cary, North Carolina) was used for all statistical analyses.

This research involved secondary analysis of deidentified data and was approved by the University of Wisconsin Institutional Review Board.

RESULTS

In unadjusted analyses, both mean maternal age and mean paternal age were significantly higher for ASD cases than for the birth cohort as a whole (Table 2). Table 2 also shows that mean parental ages differed significantly in unadjusted analyses across categories of birth order, maternal education, ethnicity, multiple birth, gestational age, and birth weight for gestational age, but not for gender. With parental age 25–29 years as the reference group, the odds of developing ASD was significantly reduced for parental age <20 years and increased for maternal age ≥35 and paternal age ≥40 years (Table 3, unadjusted odds ratios). We therefore used these age cutoffs (maternal age ≥35, paternal age ≥40 years) to classify each parent's age as "older" versus "younger." Other significant predictors of ASD in unadjusted analyses included low birth order, male gender, advanced maternal education, and preterm birth (Table 3).

Multivariable analysis of parental ages modeled as categorical variables

After we adjusted for the other parent's age and other covariates, the increases in ASD risk associated with maternal age ≥35 years and paternal age ≥40 years (relative to age 25–29 years) were slightly reduced compared with the unadjusted analysis (Table 3). In contrast, the results for birth order suggest that the decline in ASD risk associated with increasing birth order is somewhat stronger in the adjusted analysis than in the unadjusted analysis (Table 3). In addition, the apparent increase in ASD risk associated with higher levels of maternal education in the unadjusted analysis is no longer evident in the adjusted model, suggesting that the apparent maternal education effect is due to its association with parental age (Table 3).

Parental ages modeled as continuous variables

In unadjusted analyses, the risk of developing ASD increased significantly with each 10-year increase in both maternal age and paternal age. After adjustment for age of the
Table 2. Unadjusted Mean Maternal and Paternal Ages at Delivery for ASD Cases Compared With the Cohort as a Whole, and in the Cohort as a Whole Stratified by Covariate Categories, 1994 Birth Cohort From 10 Study Sites From the US Centers for Disease Control and Prevention’s Autism and Developmental Disabilities Monitoring Network

<table>
<thead>
<tr>
<th>ASD case status</th>
<th>No.</th>
<th>%</th>
<th>Mean Age, years</th>
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<td>Maternal</td>
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<tr>
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<td>54.7</td>
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<td>Birth order</td>
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<td>41.9</td>
<td>26.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>88,065</td>
<td>34.6</td>
<td>28.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>27.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>226,128</td>
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<td>28.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&gt;41</td>
<td>6,252</td>
<td>2.5</td>
<td>27.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Birth weight for gestational age&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>&gt;2 SDs below the mean</td>
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<td>1.7</td>
<td>27.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1–2 SDs below the mean</td>
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<td>11.9</td>
<td>27.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Within 1 SD of the mean</td>
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<td>71.1</td>
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<tr>
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<td>12.5</td>
<td>28.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>&gt;2 SDs above the mean</td>
<td>7,254</td>
<td>2.9</td>
<td>29.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, autism spectrum disorder; SD, standard deviation.
<sup>a</sup> Analysis of variance across all strata for this variable *P* < 0.0001.
<sup>b</sup> Number of standard deviations from the mean birth weight at a given gestational age for each gender based on all 1994 US births.

other parent and other covariates, each 10-year increase in maternal age was associated with a 20% increase in ASD risk (odds ratio = 1.2, 95% confidence interval: 1.1, 1.4) while each 10-year increase in paternal age was associated with a 30% increase in ASD risk (odds ratio = 1.3, 95% confidence interval: 1.1, 1.5).

**Combined effects of parental age and birth order**

The risk of ASD within each of 3 parental age categories (both parents “younger,” 1 parent “older,” and both parents “older”) was highest among firstborn children and declined with increasing birth order (Table 4). Considering the
<table>
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<tr>
<th>Maternal age at delivery, years</th>
<th>ASD Cases No.</th>
<th>ASD Cases %</th>
<th>Birth Cohort Comparison Group No.</th>
<th>Birth Cohort Comparison Group %</th>
<th>Unadjusted OR</th>
<th>95% CI</th>
<th>Maternal Age at Delivery, years</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
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</thead>
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<td>&lt;20</td>
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<td>21,507</td>
<td>8.5</td>
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<td>0.5, 0.8</td>
<td>0.7a</td>
<td>0.5, 1.0</td>
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<td>0.7, 1.0</td>
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<td>0.8, 1.1</td>
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<tr>
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<td>1.4</td>
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<td>Paternal age at delivery, years</td>
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<td>0.7, 1.1</td>
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<td>0.9, 1.3</td>
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<td>1.0</td>
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<td>0.7, 0.9</td>
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<td>0.8b</td>
<td>0.5, 0.8</td>
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<td>3.7, 4.9</td>
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<tr>
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<td>0.9, 1.2</td>
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<td>Child's race/ethnicity</td>
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<td>1.4b</td>
<td>1.2, 1.7</td>
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<td>Reference</td>
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<td>6,218</td>
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<td>0.8, 1.6</td>
<td>1.1</td>
<td>0.8, 1.5</td>
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<tr>
<td>Birth weight for gestational age</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>2 SDs below the mean</td>
<td>22</td>
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<td>4,223</td>
<td>1.7</td>
<td>1.1</td>
<td>0.7, 1.6</td>
<td>1.1</td>
<td>0.7, 1.6</td>
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<tr>
<td>1–2 SDs below the mean</td>
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<td>30,172</td>
<td>11.9</td>
<td>1.1</td>
<td>0.9, 1.2</td>
<td>1.1</td>
<td>0.9, 1.3</td>
<td></td>
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<tr>
<td>Within 1 SD of the mean</td>
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<td>69.9</td>
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<td>71.1</td>
<td>1.0</td>
<td>Reference</td>
<td>1.0</td>
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<td>1–2 SDs above the mean</td>
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<td>1.0</td>
<td>0.8, 1.2</td>
<td>1.0</td>
<td>0.9, 1.3</td>
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</tr>
<tr>
<td>&gt;2 SDs above the mean</td>
<td>41</td>
<td>3.3</td>
<td>7,213</td>
<td>2.9</td>
<td>1.2</td>
<td>0.9, 1.6</td>
<td>1.3</td>
<td>0.9, 1.6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; SD, standard deviation.

* Adjusted for all other variables in this column in addition to site indicators.

b Odds ratios with confidence intervals that exclude 1.0.

c Because the increased risk was similar for ages 35–39 and ≥40 years, the high-risk maternal age category was defined as ≥35 years.
Table 4. Adjusted Odds Ratios\textsuperscript{a} With 95\% Confidence Intervals Indicating Increasing Risk of ASD With Parental Age\textsuperscript{b} and Decreasing Risk With Birth Order, 1994 Birth Cohort From 10 Study Sites From the US Centers for Disease Control and Prevention's Autism and Developmental Disabilities Monitoring Network

<table>
<thead>
<tr>
<th>Birth Order</th>
<th>Both Parents Younger (Mother Aged 20–34 and Father Aged &lt;40 Years)</th>
<th>Only 1 Parent Older (Mother Aged ≥35 or Father Aged ≥40 Years)</th>
<th>Both Parents Older (Mother Aged ≥35 and Father Aged ≥40 Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% of Total Cohort</td>
<td>OR</td>
</tr>
<tr>
<td>1st</td>
<td>77,883</td>
<td>33.4</td>
<td>1.7\textsuperscript{c}</td>
</tr>
<tr>
<td>2nd</td>
<td>70,123</td>
<td>30.1</td>
<td>1.4\textsuperscript{d}</td>
</tr>
<tr>
<td>≥3rd</td>
<td>44,329</td>
<td>19.0</td>
<td>1.0 Reference</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio.
\textsuperscript{a} Adjusted for gender, gestational age, birth weight for gestational age, multiple birth, maternal ethnicity and education, and site indicators.
\textsuperscript{b} Births to mothers aged <20 years were excluded.
\textsuperscript{c} Odds ratios with confidence intervals that exclude 1.0.

The combined effects of parental age and birth order, we excluded from the analysis births to mothers aged <20 years and found the lowest risk group to be third- or later-born offspring of mothers aged 20–34 years and fathers aged <40 years. Compared with that for this group, the risk of ASD increased with both declining birth order and increasing number of older parents. The highest risk group included firstborn offspring of mothers aged ≥35 years and fathers aged ≥40 years, with a risk 3 times that of the reference group (Table 4).

DISCUSSION

Our findings are consistent with those recently reported from a large study of members of a California health maintenance organization (4) that found the risk of ASD to be positively and independently associated with both maternal and paternal age, with adjusted odds ratios nearly identical to those reported here. These findings contrast somewhat with 5 other recent epidemiologic studies that found only 1 or neither parent's age to be associated with ASD risk after controlling for the other parent's age (2, 3, 12–14).

The lack of consistency across studies could be due to limitations of sample size and of population representation of previous studies as well as other methodological differences, including autism case definitions and inclusion criteria and the ability to control for important variables. The present study included a large sample of children with sufficient information to enable evaluation of separate and combined effects of each parent's age as well as birth order and other variables. With more than 1,200 cases, it included over 50\% more cases and thus more statistical power than any of the previous studies examining independent effects of maternal and paternal age on ASD risk.

Another advantage of this study is the population-based nature and diversity of the cohort, allowing control for factors that may confound the association between parental age and ASD. Maternal education is 1 variable we considered to be a potentially confounding factor because it is associated with maternal age and has been observed to be related to ASD risk (15). Our results, however, suggest that the association between advanced maternal education and ASD risk observed in unadjusted analysis may be spurious and due to confounding by parental age.

The results of this study also demonstrate the importance of controlling for birth order in evaluating independent effects of parental age on ASD risk. Because birth order increases with parental age and, in this and other studies, has been found to be negatively associated with ASD risk, failure to control for birth order may mask a positive association between parental age and ASD risk. Two of the previous studies reporting an association between advancing maternal age and ASD (2, 4) also had adjusted for birth order and, similar to the present study, found birth order to be negatively associated with ASD.

An additional advantage of this study is its restriction to a single birth year, thereby controlling for temporal trends in recent decades in both ASD prevalence and parental ages at the birth of their children. This feature of the study allows estimation of the association between parental age and ASD risk independently of temporal trends in diagnostic practices or other factors.

Public health implications

The strength of the independent associations between maternal and paternal age and ASD risk, as indicated by the odds ratios in the range of 1.2–1.4 reported here, is modest. However, the observation that these effects are independent of each other and of low birth order raises the likelihood that the combined effects of parental age and birth order may have important public health implications. Mean maternal age in the United States has increased steadily since the 1970s, particularly for firstborn children, for whom mean maternal age at delivery increased by 3.8 years between 1970 and 2004 (16). In addition, the proportion of births to women aged ≥35 years began increasing in the United States after 1980, when it was 5\%; by 2004, it had increased to 14.2\% (17, 18). During this same period, fertility rates for men aged ≥40 years also increased each year, while fertility among men aged <30 years declined (16). With the decline in average family size in recent decades, we would also expect the proportion of children who are firstborn to have increased. Similar trends are occurring in other developed countries (7). The results of this study raise the question of whether some portion of the recent rise in ASD prevalence (19) may be linked to recent trends in...
parental age and family size. A further question is whether a modest increase in prevalence associated with advancing parental age and low birth order may have contributed to a greater awareness of ASD and, in turn, increases in measured prevalence. The tendency for older parents of firstborn children to have higher levels of educational achievement and resources than other parents could further contribute to increased awareness and an expansion of the diagnosis of ASD.

**Potential etiologic implications of parental age effects**

Because we observed independent effects of the age of each parent on ASD risk, the possible mechanisms for these effects could include a broad range of processes associated with either or both maternal and paternal age. The observed paternal age effect independent of maternal age could point to a causal role of gene mutations in male germ cells, because the probability or selection of these mutations increases as men age (20, 21). The independent effect of maternal age, on the other hand, may point to age-related chromosomal changes, pregnancy complications, or environmental exposures during pregnancy. Independent effects of 1 or both parents’ ages also could point to a role of accumulated environmental exposures that may have mutagenic effects on gametes or could result from a combination of mechanisms (21, 22).

The association between advanced maternal and paternal age and ASD is also consistent with a potential role of infertility treatments or assisted reproductive technologies, the uses of which have increased in the past decade, especially by women and men of advanced reproductive age (23). Numerous studies have found associations between these technologies and adverse pregnancy outcomes, including those due to epigenetic effects (24–27), although a recent review found no evidence of elevated rates of autism among children born after in vitro fertilization techniques (28). Even though we have no information about exposure to these treatments in our cohort, the observation that firstborn children of older parents had the highest ASD risk is consistent with a possible infertility treatment effect because women who give birth after infertility treatment are more likely to be primiparous than those represented in the general birth cohort. However, the association between multiple birth and ASD in this study was weak and not statistically significant (Table 3, unadjusted odds ratio), whereas assisted reproduction technologies are strongly associated with multiple birth (23).

Another unmeasured factor in the present study potentially associated with both advanced parental age and ASD risk in offspring is psychopathology or behavioral traits of parents that may result in both delayed parenthood and genetic susceptibility to autism in offspring (14).

**Birth-order effects**

The observation in this and at least 2 previous studies (2, 4) that the risk of developing ASD was highest for firstborn children and declined with increasing birth order is a pattern also observed for other childhood disorders, including type 1 diabetes and atopy, and is cited as support for the “hygiene hypothesis.” According to this hypothesis, firstborn children are exposed to fewer infections from other children early in childhood and, because of delayed immunologic challenge, may be more likely to develop autoimmune responses including those that may adversely affect neurodevelopment (29). Another possible factor that could lead to the observed birth-order effect is exposure to potentially neurotoxic, fat-soluble chemicals accumulated in maternal tissue that have been passed to offspring transplacentally or through breast milk (30). Because of accumulation over a lifetime, the load of such neurotoxins transmitted might be expected to be highest for firstborn children, particularly when combined with advanced maternal age. Another possible explanation for the observed birth order effect is “stoppage” or a tendency for parents of 1 child with ASD not to have subsequent children because of the demands of parenting a child with a disability or concerns about genetic susceptibility (31), thus increasing the likelihood in the cohort as a whole that a child with ASD will have a low birth order. Information available for the present study did not allow examination of these hypotheses.

Another important limitation of this study is that the cohort available for analysis excludes births with missing paternal age information. Because this exclusion applied to both the ASD cases and the comparison group (Table 1), we would not expect it to have resulted in biased estimates of the association between ASD and parental age. In a separate analysis, we examined the association between maternal age and ASD without adjusting for paternal age and including the full birth cohort, and we found the association between maternal age and ASD to be the same as that observed in the subcohort with paternal age.

Another limitation is that the birth cohort comparison group includes about 1% of births of children who died postnatally in addition to an undetermined number who moved out of the study area between birth and the age of 8 years, whereas children who died postnatally and those moving out of the study area after birth are excluded from the case group. Because of this limitation, we could not estimate cumulative incidence of ASD. Nonetheless, this limitation is unlikely to have biased the estimated odds ratios reported in this study, particularly those adjusted for factors such as gestational age and birth weight for gestational age, which are strongly associated with postnatal mortality. Another possible explanation for the increase in ASD among offspring of older parents, but one we cannot evaluate with the data available, is that, compared with younger parents, older parents may be more aware of developmental abnormalities and better able to access diagnostic and special educational services. Other limitations are that parity pertains to only mothers and does not take into account the number of previous births fathered by the fathers represented in the cohort, potential for residual confounding by factors not measured in the present study, possible misclassification of ASD case status, and missing information on paternal education.

**Conclusion**

The results of this study provide the most compelling evidence to date that ASD risk increases with both maternal and paternal age and decreases with birth order. Further
research involving large, well-characterized birth cohorts followed longitudinally will be required to confirm these findings and adequately evaluate the range of alternative genetic and environmental hypotheses that this and other studies raise regarding parental age and birth-order effects on ASD risk. Smaller, focused studies may also be useful, such as Crow's idea to look for mutations responsible for complex disorders of unknown etiology and with parental age effects by studying affected families with older parents (20).

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PAPERS TO BE SUBMITTED FOR PUBLICATION THAT WERE GENERATED BY 2007 COUNCIL FUNDING
Stathmin Reveals Opposing Roles of Basolateral Amygdala in Affiliative and Social Interactions

Guillaume Martel, Akinori Nishi, Gleb P. Shumyatsky

Materials and methods

Mice
Stathmin\textsuperscript{-/-} mice were maintained on C57BL/6J background (N>10). For the lesion studies, wildtype C57BL/6J mice were used. All mice were maintained on a 12 h light/dark cycle. Behavioral experiments were conducted during the light phase of the cycle, and mice (except for pups) were at least 12 weeks old at the time of training.

Amygdala lesions
Brain lesions were performed using standard stereotaxic procedures. Flat skull stereotaxic coordinates were derived from the atlas (1) using bregma of the skull surface as the reference point. The coordinates for amygdala lesions were 2.0 mm anterior, 3.25 mm lateral and 4.3 mm ventral. Lesions were made by injecting 0.3 \mu l of ibotenic acid with injection cannulae (0.2 mm diameter and 9 mm long) linked to 1 \mu l Hamilton syringes via polyethylene catheter tubing. The syringes were held in a constant-rate infusion pump and bilateral injections were conducted over a 3 min period. In all cases, correct injection flow rates were visually controlled. The cannulae were left in place for additional 2 min before removal. Sham-operated animals received the same surgical treatment but were injected with 0.3 \mu l of artificial cerebrospinal fluid. A recovery period of 10 days was given after the surgery. Amygdala lesions were performed on 29 mice total (10 sham-lesioned animals and 19 BLA-lesioned animals). After histological evaluation of lesion localization, 7 mice were excluded from the behavioral analysis. Figure 5 (fig. S5) in Supplemental Material shows the spreading of the amygdala lesions.

Behavioral experiments

For all behavioral experiments, females were housed individually one week before the experiment with the nest material placed in the cage. Each behavior was videotaped with minimal disturbance and analyzed offline later by an observer blind to the genotype.

Pup retrieval in the home cage

On the day of the experiment, 3 pups (foster pups for virgin females and female’s own pups for postpartum females) were placed in three corners away from the nest in the home cage. Each mouse was tested for 20 min. The latencies to sniff and retrieve each pup were recorded. Retrieval was defined as a female picking up a pup in her mouth and transporting it to the nest. 22
wildtype mice, 21 *stathmin*+/− mice, 8 sham- and 12 amygdala-lesioned mice were used. 2 sham-lesioned mice were excluded due to infanticide.

**Rescue of the pup retrieval deficit**

The procedure was similar to that described above for pup retrieval in the home cage. However, before the experiment pups were placed for 5 min in the nest of a virgin female to be tested (with the female present). Immediately or one hour after the 5-min pup exposure, the 3 pups were removed from the nest and placed in three corners of the same cage and pup retrieval was recorded as described for the procedure of pup retrieval in the home cage. 7 wildtype females and 10 *stathmin*+/− mice were used to test the rescue of pup retrieval deficit immediately after the pup exposure. For the rescue of the pup retrieval deficit one hour after the pup exposure, 11 wildtype and 11 *stathmin*+/− mice were used.

**Olfactory function**

A filter paper (1.2cm x 1.2cm) was attached on the wall of female home cage. After 5 min habituation to the filter paper, 10 μl of distilled water was applied to the filter paper. The sniffing to the distilled water was measured for 5 min. One day after this testing, the same procedure was used to test male urine. 14 wildtype females and 11 *stathmin*+/− mice were used.

**Nesting behavior**

A piece of cotton (0.6 g) was placed in the home cage of each female. After 90 min and 24 h, the nesting behavior was scored as no nesting (0), nesting not complete (1) and complete nesting (2). For this experiment, 12 wildtype females and 12 *stathmin*+/− mice were used.

**Postpartum maternal care**

**Standard breeding**

Fifty females (25 wildtype and 25 *stathmin*+/− mice) were housed with wildtype males. All females were housed individually once pregnant. Births were recorded each morning. For a period of 21 days following delivery, the number of pups born, individual pup weight, and the number of pups survived were measured. After 21 days, pups were removed from the cage and a new male was introduced into the cage; it was removed when the female became pregnant (at approximately 14 days).

**Interchange of litters between wildtype and knockout females**

Thirty females (15 wildtype and 15 *stathmin*+/− mice) were housed with wildtype males. All females were housed individually once pregnant. On the day of the delivery, pups born by wildtype females were given to *stathmin*+/− females and pups born by *stathmin*+/− females were given to wildtype females. For a period of 21 days following delivery the number of pups born, individual pup weight, and the number of pups survived were measured.

**Pup retrieval in the open field**
Females were already pre-exposed to the open field to reduce their exploratory behavior and were experienced in pup retrieval so that the initial deficit in pup retrieval in the knockout mice will not affect their strategy. The nest, previously made by the female, was broken apart and spread evenly throughout the open field. Three pups were placed in each of the three positions: in the corner, in the middle of the wall, and in the center of the arena. Then the female was introduced and observed for place preference in nest building and pup retrieval. All mice that were used re-built the nest and retrieved the pups in the nest at the end of the experiment. For this experiment, 14 wildtype females, 12 \textit{stathmin}^{+} mice, 7 sham- and 12 amygdala-lesioned mice were used. 3 sham-lesioned females were excluded due to infanticide.

\textit{Open field}

Mouse was placed in the corner of the open field (43.2cm x 43.2cm) and observed for 20 min. The time spent in the periphery and in the center of the arena was recorded using an automated tracking system (Open Field Activity Software, Med Associates). Results are expressed as the ratio of the time spent in the center over the time spent in the periphery. 10 wildtype mice, 10 \textit{stathmin}^{+} females, 10 sham- and 12 amygdala-lesioned mice were used.

\textit{Elevated plus maze}

Elevated plus maze (1 meter above the floor) consists of a center platform (10 cm x 10 cm), two open arms (40 cm x 10 cm) and two closed arms (40 cm x 10 cm) within walls (30 cm high). A mouse was placed in the center of the apparatus and the time spent in each arm was measured during a 5-min interval using Limelight software (Coulbourn Instruments). For this experiment 11 wildtype mice, 11 \textit{stathmin}^{+} mice, 9 sham- and 12 amygdala-lesioned mice were used. One sham-lesioned female was excluded because she fell from the apparatus.

\textit{Social interaction}

Females were individually housed one week before testing. On the day of the test, an intruder female was placed in the home cage of the host female for 15 min and interactions between females were video recorded. 10 wildtype mice, 9 \textit{stathmin}^{+} females, 10 sham- and 10 amygdala-lesioned females were used. Two amygdala-lesioned mice were excluded because the host or the intruder escaped during the experiment.

\textit{Hoarding behavior}

Mice were housed in plastic cages with wood shaving bedding. Each cage was connected to an external plastic tube (4.8 cm in diameter, 37 cm long) at the far end of which 50 g normal diet food pellets (each around 1.7g) were placed. Water and 2 g of food were provided for each cage. Mice and their nests were individually placed in the cages early in the morning of the test day for habituation with the hoarding tube entrances blocked. The remaining food in the cages was removed and the tubes were opened in the late afternoon, just before
the start of the dark phase. Next morning the pellets remaining in each tube were weighed and the difference in weight between the start and end of the test was considered, for practical purposes, to be the weight of food hoarded. 8 females of both genotypes were used for this experiment.

Swim forced test

Mice were placed individually into glass cylinders (height 30 cm, diameter 15 cm) containing 15 cm of water, maintained at 23–25°C. The animals were left in the cylinder for 5 min. The total duration of immobility was measured during the 5-min test. The mouse was judged to be immobile when it remained floating passively in the water. 10 wildtype mice and 13 stathmin females were used for this experiment.

In situ hybridization

RNA in situ hybridization was performed as described previously (2).

Immunohistochemistry

24 virgin female mice (12 wildtype and 12 stathmin females), 2 to 3 months old were used. 16 mice (8 of each genotype) were subjected to pup retrieval testing as described above and 8 mice (4 of each genotype) were used naïve. One hour later, mice were perfused transcardially with ice-cold solutions of 4% paraformaldehyde in phosphate buffer (PBS; 0.1 M, pH 7.4). After post-fixation overnight in the same fixative at 4°C, coronal sections (40 μm) were cut on a vibratome and collected in PBS Buffer.

C-fos Immunohistochemistry

After elimination of endogenous peroxidase activity and a preincubation step, sections were incubated for 24 h with rabbit anti-c-Fos antibody (1:10000 dilution; Calbiochem). Subsequently, sections were incubated with biotinylated goat anti-rabbit antibody (1:600; Vector ABC kit) and with the ABC complex (Vector ABC kit) and staining was visualized with diaminobenzidine (DAB). Sections were mounted on gelatin-coated slides, air-dried, dehydrated, covered with a glass coverslip using Gel-Mount (Biomedica) and examined using light microscopy.

Oxytocin immunohistochemistry

After elimination of endogenous peroxidase activity and a preincubation step, sections were incubated for 24 h with rabbit anti-oxytocin antibody (1:5000 dilution; Santa-Cruz). Subsequently, sections were incubated with biotinylated goat anti-rabbit antibody (1:200; Vector ABC kit) and with the ABC complex (Vector ABC kit). Staining was visualized with DAB.

Quantification and analysis

The number of positive cells was counted in MPA, amygdala (LA and BA), and PVN using ImageJ (NIH). Nuclei were counted and expressed as the number of positive nuclei per square mm.
Statistical analysis

Statistical analyses were run by way of SAS (SAS Institute, Cary, NC, USA) and Systat 9 software. Statistical analyses of immunohistochemical data and behavioral experiments were performed using ANOVAs and subsequent post hoc tests (Scheffe F test).

For the assessment of the nest location during pup retrieval in the open field, statistical analyses were run by way of Systat 9 software. Chi-square (χ2) analyses with Yates corrections (a conservative adjustment allowing comparisons of cells with frequencies of less than five) were conducted to determine if any genotype exhibited a significant tendency to build the nest in a particular location. Within-group comparison between the percentages of each location used paired t-tests to determine which location type was preferred.
Fig. S1. Olfaction, organizational skills and the general level of motivation are normal in \textit{stathmin}\textsuperscript{+/+} females. (A) The amount of time spent sniffing male urine and water is normal in \textit{stathmin}\textsuperscript{+/+} females. (B) Organizational skills as tested by hoarding behavior are normal in \textit{stathmin}\textsuperscript{+/+} females. (C) The general level of motivation as analyzed in Porsolt forced swim test is normal in \textit{stathmin}\textsuperscript{+/+} females. Results are presented as mean ± SEM.
Fig. S2. Pups survival is reduced in litters of *stathmin* females. (Left) Percentage of pups dead over the period from the delivery day until 21 days after the delivery. (Right) Number of pups born by wildtype and *stathmin* mothers. Results are presented as mean ± SEM. * represents *P < 0.05.*
Fig. S3. Weight of pups born and raised by postpartum *stathmin-/-* and wildtype females. (Top) Weight of pups over the 21 days of the experiment. (Bottom) First 8 days of the experiment. Results are presented as mean ± SEM.
Fig. S4. Weight of pups born by wildtype females and raised by stathmin$^{-/-}$ mothers and weight of pups born by stathmin$^{-/-}$ females and raised by wildtype mothers. (Top) Weight of pups over the 21 days of the experiment. (Bottom) First 8 days of the experiment. Results are presented as mean ± SEM.
Fig. S5. Histological evaluation of BLA lesion localization. (A-L) Brain atlas schemes show the size of the lesions in the brain, going from (A) anterior to (L) posterior sections. Grey color represents the smallest lesion and blue color represents the largest lesion.
Fig. S6. Representative pictures showing immunohistochemistry for Fos and oxytocin. (A, B, C and D) Fos staining. (E, F, G and H) oxytocin staining. Pictures show expression in wildtype (A, C, E, and G) and stathmin^+^ females (B, D, F and H) in the amygdala (A and B), MPA (C, D, G and H) and PVN (E and F).
References and Notes


Stathmin Reveals Opposing Roles of Basolateral Amygdala in Innate Behaviors Essential for Survival

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One-sentence summary: A gene knockout unravels contrasting differences in how the amygdala is required for strengthening parental behaviors and at the same time inhibiting adult social interactions.

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Abstract

Behaviors involving risk assessment are strongly modulated by the amygdala-associated circuitry via mechanisms that are still unclear. Mice without an inhibitor of microtubules stathmin, enriched in the basolateral amygdala (BLA), are deficient in fear processing. We here report that this deficit leads to improper risk assessment, which in turn affects in opposing directions affiliative maternal care and adult social interactions in \( stathmin^{+/−} \) females. Profound deficiency is observed in the maternal behavior of \( stathmin^{-/−} \) females: they lack motivation for retrieving pups and are unable to choose a safe location for the nest. In contrast, \( stathmin^{+/−} \) females show a remarkable enhancement in social interactions. BLA lesions in wildtype mice produce similar effects in maternal and social behaviors. Our findings pinpoint stathmin as the critical molecular component, involved in BLA function as a detector of danger in behaviors essential for survival.
Among Niko Tinbergen's four "whys", representing a framework for understanding animal behavior, the third "why" refers to the survival value of a behavior. The behaviors that are essential for survival of the individual along with the species as a whole (1, 2) require risk assessment of the environment and are regulated by the amygdala-associated neural circuitry of fear (3). The amygdala is not a single anatomic and functional unit, and nuclei of the amygdala have multiple inter- and intra-connections (4-6). Moreover, the amygdala nuclei are involved in various fear-related and reward-related behaviors (7-11). Unraveling the molecular, cellular and anatomic mechanisms underlying the relationship between amygdala-associated circuitry and other behaviors critical for survival is necessary to our understanding of how risk assessment controls behavioral choices in animals and humans. The BLA plays a key role in memory for learned fear. However, its role in innate behaviors is not well understood. Here we examine the role of the BLA in two behaviors vital for individual and species survival: affiliative maternal care and adult social interactions. During maternal behavior, failure to keep progeny away from danger can be detrimental to their well-being. Also, risk assessment during social interactions is essential for the establishment of social hierarchy. The amygdala-mediated ability to detect danger is expected to have a strong impact on the ultimate success of these behaviors. To examine the impact of BLA dysfunction, we employ two approaches: mice with BLA lesions and mice deficient in BLA-enriched gene stathmin, which were previously shown to have specific deficits in innate and learned fear.

First we examined stathmin<sup>−/−</sup> mice in pup retrieval, which represents a motivational aspect of maternal care (12). Virgin (nulliparous) laboratory mice inexperienced in pup retrieval immediately engage in pup retrieval behavior when exposed to foster pups in
their home cage (13). Pup retrieval was tested by placing three foster pups in different corners of the home cage of a virgin female and monitoring the latency of the female retrieval of the pups to the nest (14). Statmin\(^{−/−}\) females showed a significant deficit in pup retrieval on the first (Fig. 1A; \( P = 0.001\)) and second (Fig. 1B; \( P < 0.001\)) days. The latency of pup approach and sniffing was normal (Fig. 1C; \( P = 0.879\)), indicating that the females were aware of the presence of the pups. Other related functions such as nest building (Fig. 1D; \( P = 0.374\)), olfaction (fig. S1A; \( P = 0.776\)), and hoarding (fig. S1B; \( P = 0.345\)) were normal, suggesting the specificity of the maternal phenotype. Similarly, we have found deficits in pup retrieval in post-partum statmin\(^{−/−}\) females in both their first and second litters (Figs. 1E and 1F; \( P_s < 0.05\)). The number of pups born and the pups' survival rate from day 1 to day 21 were decreased in the litters of statmin\(^{−/−}\) females (fig. S2; \( P_s < 0.05\)). Moreover, the weight of pups raised by statmin\(^{−/−}\) females was decreased for the first eight days compared to pups raised by the wildtype females, irrespectively of the progeny genotype (figs. S3-S4; interaction between days and genotype, \( P < 0.05\)).

Using RNA in situ hybridization (14), we have analyzed statmin expression in the anatomic areas responsible for maternal care (13). Compared to the lateral (LA) and basal (BA) nuclei of the amygdala (throughout the text we refer to these two nuclei as the basolateral complex, BLA; Fig. 2A), statmin RNA was negligible in the major areas involved in pup retrieval, including the medial preoptic area (MPA), bed nucleus of the stria terminalis (BST), medial nucleus of the amygdala (MeA) and other related regions (Figs. 2A and 2B). We hypothesized that statmin action in the BLA is required for normal pup retrieval. Using ibotenic acid, we made bilateral lesions (14) in the BLA in virgin wildtype females (fig. S5) and tested them for pup retrieval in the home cage. Pup
retrieval was deficient in the lesioned animals (Fig. 1A; $P < 0.05$), confirming the involvement of the BLA in this behavior.

Remarkably, the pup retrieval deficit can be completely rescued (I4) by a pre-exposure of stathmin\textsuperscript{+/} virgin females to foster pups for five minutes in the female’s nest immediately before testing (Fig. 1G; $P = 0.996$). A one-hour delay, on the other hand, does not rescue this deficit (Fig. 1H; $P < 0.001$). This short-term recovery strongly suggests that only the motivational aspect of maternal behavior is abnormal in virgin stathmin\textsuperscript{+/} mice. This finding is consistent with a notion that the BLA is involved in the motivational aspects of behavior (I5-I7). To determine whether the decrease in motivation in pup retrieval is specific to maternal care or reflects a general increase in depression in stathmin\textsuperscript{+/} females, we examined their performance in the Porsolt forced swim test, which is an accepted measurement of depression (I8). We found no differences in performance of this task (I4) between the mutants and wildtype mice (fig. S1C, $P = 0.395$), confirming that the decrease in motivation is specific to pup retrieval.

Using Fos as a marker on neuronal activity (I3), we examined the amygdala as well as the anatomic areas responsible for maternal care (I3) in stathmin\textsuperscript{+/} females following pup retrieval in the home cage (I4). Fos staining was comparable in the MPA in both the mutant and control mice (Fig. 2E and fig. S6; $P > 0.1$), as expected, since stathmin is not normally expressed in this area (Fig. 2B). This suggests that the pup retrieval deficit does not originate in the MPA dysfunction in stathmin\textsuperscript{+/} mice, which is consistent with the finding that the deficit can be rescued (Fig. 1G). However, Fos induction was significantly reduced in the lateral and basal nuclei of the amygdala in stathmin\textsuperscript{+/} mice compared to control mice (Figs. 2C, $P < 0.05$; Fig. 2D, $P < 0.01$), confirming our
hypothesis that the amygdala is involved, given its known role in motivation (7, 19) and the processing of fearful events (9). The Fos experiments suggest that the BLA is involved in pup retrieval and that its function is impaired in stathmin
-/- mice. Because a number of studies have shown that oxytocin plays an important role in maternal behavior (13, 20), we also tested expression of this neuropeptide. No differences were found in oxytocin expression between the mutant and wildtype littermates in the MPA and paraventricular hypothalamic nucleus (PVN) (Figs. 2F and 2G; \( P_s > 0.43 \)). This was observed both in naïve animals and following pup retrieval. Interestingly, this suggests that stathmin modulates pup retrieval behavior via mechanisms that do not involve oxytocin function.

To examine further the importance of stathmin for maternal behavior, we tested stathmin
-/- females in pup retrieval in an anxiogenic environment. First, the basal anxiety levels were determined in stathmin
-/- females using the open field and elevated plus maze. The mutant females showed strong deficits in innate fear compared to the wildtype controls, as they spent significantly more time both in the center of the open field (Fig. 3A, \( P < 0.0001 \)) as well as in the open arms of the elevated plus maze (Fig. 3B; \( P = 0.001 \)), confirming earlier results found in stathmin
-/- males (21). Next, we investigated maternal care in the presence of a potential threat by developing a paradigm (14) to test pup retrieval in the open field (Fig. 3C). As expected, the wildtype mice preferred safe locations for the nest: the majority selected a corner (76.2%) and the rest chose the middle of the wall (19%) or the center of the arena (4.8%), which is the most exposed and dangerous place for pups in the open field (Fig. 3C; \( P < 0.01 \)). In a striking difference,
*stathmin*<sup>−/−</sup> mice selected at random either the corner, the middle of the wall, or the center of the arena (Fig. 3C; $P > 0.166$).

To confirm that the maternal care deficits in *stathmin*<sup>−/−</sup> mice are caused by the dysfunction of the BLA, we examined wildtype females with ibotenic acid-induced bilateral lesions to the BLA. The lesions caused a decrease in innate fear in the open field (Fig. 3A, $P < 0.05$) and in the elevated plus maze (Fig. 3B, $P < 0.001$), confirming previous studies (22). Pup retrieval in the open field by the BLA-lesioned wildtype mice showed the same random pattern as observed with *stathmin*<sup>−/−</sup> mice (Fig. 3C; $P > 0.166$).

To test another behavior critical for individual survival, we turned to social interactions and examined adult *stathmin*<sup>−/−</sup> females in the host-intruder paradigm (14). In contrast to the deficit in maternal behavior, *stathmin*<sup>−/−</sup> mice demonstrated an increase in female-female social interactions (Fig. 4A; $P < 0.001$). They spent significantly more time following an intruder female around the cage; no aggressive behaviors were observed. It is important to note that object recognition was normal in *stathmin*<sup>−/−</sup> mice (Fig. 4B; $P = 0.290$), suggesting a specificity of the phenotype to social interactions. To examine the contribution of the BLA to the social abnormality found in *stathmin*<sup>−/−</sup> females, the BLA-lesioned wildtype females were also subjected to the host-intruder paradigm. Similar to *stathmin*<sup>−/−</sup> females, the wildtype females with amygdala lesions showed an increase in social interactions compared to the control animals (Fig. 4A; $P < 0.01$).

These findings reveal several major points. First, they expose new functional connections of the BLA which allow regulation in opposing directions of two behaviors essential for survival, maternal care and social interactions (Fig. 4C). Second, this work
provides genetic evidence that stathmin is required for this function (Fig. 4C). Third, the BLA-associated neural circuitry expressing stathmin is involved in behaviors vital for survival via the element of risk assessment. Moreover, the fact that maternal behavior and social interaction are affected in opposing directions suggests that both stathmin and the BLA modulate these behaviors with high specificity. This is consistent with the previous work that has shown that \textit{stathmin}^{-/-} mice are deficient in amygdala-dependent innate and learned fear, but are normal in hippocampus-dependent spatial memory tested in the water maze (21). At the cellular level, stathmin is a negative regulator of microtubule formation (23) and in \textit{stathmin}^{-/-} mice an increase in the microtubule stability in the amygdala leads to deficits in amygdala synaptic plasticity (21), suggesting that the deficiency at BLA synapses may be responsible for effects described here.

Our findings suggest a clear distinction between the roles of the different nuclei of the amygdala in maternal behavior. In contrast to the inhibitory function of the MeA (13, 24, 25), our work illustrates the positive involvement of the BLA in maternal behavior. The maternal deficit that we have observed in \textit{stathmin}^{-/-} mice is also different from that in other mutant mice described so far (13, 25, 26). In contrast to the other mutant mice deficient in pup retrieval, \textit{stathmin}^{-/-} virgin females can improve their performance and their deficit can be transiently rescued by a brief pre-exposure to pups. Therefore, stathmin highlights the previously unrecognized role of the BLA in maternal behavior.

These findings have interesting parallels with recent work in humans and non-human primates that suggests that the amygdala functions as a danger detector and through the control of fear modulates behaviors where an assessment of a potential threat is important (27-29). Similarly, \textit{stathmin}^{-/-} females and mice with lesions in the BLA
show an increase in social interactions, confirming the notion that the amygdala regulates social behavior via safety assessment. A large body of research has convincingly shown the essential role of the BLA in processing of sensory information related to fear and reward. It seems thus reasonable to suggest that these basic functions of the BLA allow it to control the survival behaviors described in this report. Our work demonstrates a spectrum of surprisingly contrasting roles the amygdala plays in different behaviors that are dependent on danger detection, and pinpoints stathmin as a new molecular target for controlling anxiety and decision making related to risk assessment.

**References and Notes**


14. . (Information on materials and methods is available on Science Online).


30. We thank V.Y. Bolshakov, C.R. Gallistel, K. Herrup, E.R. Kandel, L.D. Matzel, M. Numan, and M.R. Plummer for comments on the manuscript. This work was supported by the Charles and Johanna Busch Memorial Fund (G.P.S.); New Jersey Governor's Council on Autism (G.P.S.); NARSAD (G.P.S.); and JSPS (A.N.).
Fig. 1. Affiliative maternal behavior in $\textit{stathmin}^{+/-}$ females and in wildtype females with BLA lesions. (A and B) Virgin $\textit{stathmin}^{+/-}$ females and wildtype females with BLA lesions show (A) deficiency in pup retrieval on day one and (B) day two; 1st, 2nd and 3rd, retrieval of the first, second and third pups. (C) The latency of approach to sniff the pups is normal on both days in virgin $\textit{stathmin}^{+/-}$ females and in virgin wildtype females with BLA lesions; D1 and D2, first and second days. (D) Nest building is normal in $\textit{stathmin}^{+/-}$ females 90 minutes and 24 hours after the introduction of the nest material. (E and F) Pup retrieval is deficient in post-partum $\textit{stathmin}^{+/-}$ females both for the first (E) and second (F) days. Inset in (E) shows the retrieval data for the first day using a smaller scale. (G and H) The pup retrieval deficit can be rescued by placing pups for five minutes in the nest of a virgin $\textit{stathmin}^{+/-}$ female if (G) the female is tested immediately but (H) not after a one hour delay. Results are presented as mean ± SEM.

Fig. 2. Expression of $\textit{stathmin}$ in the BLA and deficiency in Fos activity in the BLA during pup retrieval. (A) $\textit{stathmin}$ is strongly expressed in the lateral (LA) and basal (BA) nuclei but not in the medial nucleus of the amygdala (MeA); opt, optical tract. (B) $\textit{stathmin}$ is not expressed in the anatomic areas directly involved in pup retrieval; MPA, medial preoptic nucleus; BST, bed nucleus of the stria terminalis; VP, ventral pallidum; aca, anterior commissure. (C and D) During pup retrieval Fos is induced (C) normally in the lateral nucleus but (D) significantly less in the basolateral nucleus in $\textit{stathmin}^{+/-}$ females compared to wildtype mice. * represents $P < 0.05$; ** represents $P < 0.01$. (E, F and G) During pup retrieval Fos is induced (E) normally in the medial preoptic area (MPA). Oxytocin is induced
normally in (F) the MPA and (G) paraventricular hypothalamic nucleus (PVN). Results are presented as mean ± SEM.

**Fig. 3.** Deficiency in innate fear leads to deficits in risk assessment during pup retrieval in *stathmin<sup>−/−</sup>* females. (A and B) Innate fear is deficient in *stathmin<sup>−/−</sup>* females and in BLA-lesioned wildtype females. In (A) the open field the mutants and lesioned animals spend more time in the center of the arena and in (B) the elevated plus maze the mutants and lesioned animals spent more time in the open arm compared to control mice. * represents *P < 0.05; *** represents *P < 0.001. (C) Pup retrieval in the open field. *Stathmin<sup>−/−</sup>* females and BLA-lesioned wildtype females choose in random fashion the place to re-build the nest and retrieve the pups in contrast to control animals that prefer corners of the open field. Upper photograph shows the view of the open field with the pieces of the nest broken apart, three pups (dashed circles) and the female at the beginning of the experiment. Three photographs in the middle show the final stage of the experiment with three possible choices that the females make for the locations of the nest and the pups. Results are presented as mean ± SEM.

**Fig. 4.** Social recognition and object recognition in *stathmin<sup>−/−</sup>* females and in BLA-lesioned wildtype females. (A) Social recognition is enhanced in *stathmin<sup>−/−</sup>* mice and in wildtype females with BLA lesions. ** represents *P < 0.01; *** represents *P < 0.001. (B) Object recognition is normal in *stathmin<sup>−/−</sup>* mice. Results are presented as mean ± SEM. (C) A model of how stathmin expression and the BLA positively regulate affiliative maternal behavior and negatively regulate adult social interactions. Results are presented as mean ± SEM.
Fig. 3
Martel et al.
C

Wildtype female mice:

Stathmin deficient BLA

inhibition → Social behavior
activation → Maternal behavior

Stathmin" and BLA-lesioned female mice:

No Stathmin deficient BLA

activation → ↑ Social behavior
inhibition → ↓ Maternal behavior
APPENDIX C

- The list of Council-funded Clinical Enhancement Centers
- Power Point slides of the Centers’ enhancement plans
THE LIST OF COUNCIL-FUNDED CLINICAL ENHANCEMENT CENTERS
The Governor’s Council for Medical Research and Treatment of Autism
2008 Clinical Enhancement Grant Recipients

The Council’s Clinical Enhancement grant program is designed to improve families’ access to services, reduce wait times for receiving developmental evaluations, and increase the number of children that can be assessed by multidisciplinary evaluation teams. The 6 grant recipients that will be funded through this $6 million grant initiative are:

<table>
<thead>
<tr>
<th>Institution</th>
<th>Principal Investigator</th>
<th>2-yr. Funding Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) The Autism Center – University of Medicine and Dentistry, New Jersey Medical School</td>
<td>Dr. Kendell Sprott</td>
<td>$1,000,000</td>
</tr>
<tr>
<td>2) Children’s Specialized Hospital – Toms River</td>
<td>Dr. Yvette Marie Janvier</td>
<td>$1,000,000</td>
</tr>
<tr>
<td>3) The Center for Neurological and Neurodevelopmental Health II</td>
<td>Dr. Mark Mintz</td>
<td>$1,000,000</td>
</tr>
<tr>
<td>4) Institute for Child Development - Hackensack University Medical Center</td>
<td>Dr. Randye Huron</td>
<td>$1,000,000</td>
</tr>
<tr>
<td>5) Hunterdon Medical Center</td>
<td>Dr. Audrey Mars</td>
<td>$695,969</td>
</tr>
<tr>
<td>6) Jersey Shore Medical Center Foundation</td>
<td>Dr. Denise Aloisio</td>
<td>$1,000,000</td>
</tr>
</tbody>
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The 6 grant recipient centers will work in a collaborative manner to contribute patient data to both the NJDHSS’ Autism Registry and to a collective database that can be used as a tool for future research studies and clinical initiatives. The goal of the Clinical Enhancement grant program is to create an integrated network of services that will ultimately enable the State of New Jersey to pursue federal funding as an Autism Center of Excellence (ACE).

As reported in February 2007 in the Centers for Disease Control and Prevention’s (CDC) study on the prevalence of autism, 1 in every 94 children in New Jersey has an Autism Spectrum Disorder (ASD), and this prevalence rate is the highest among all of the states examined in the study. In response to this situation, the Clinical Enhancement funding will allow for staff enhancements in all of the recipient centers. Hiring additional clinical and support staff will allow centers to streamlining the patient intake process, reduce wait times for multidisciplinary evaluations, and increase the availability of community-based services by offering additional workshops with parent groups. The Clinical Enhancement
funding will also enable centers to provide additional and critical outreach to allied health practitioners and school districts to educate and assist individuals to detect and identify children with ASDs.
POWER POINT SLIDES OF THE CENTERS' ENHANCEMENT PLANS
CURRENT CRCNJ STUDIES

- Autism
  - Memantine
  - Flusazetine
- Down Syndrome
  - Donepezil
- Epilepsy
  - Clobazam for Lennox-Gastaut Syndrome
  - Lacotamide for Complex Partial Seizures
  - Injectable diazepam for Acute Repetitive Seizures
- Quality of Life
- Migraines in Adolescents
- ADHD
  - Atomoxetine
  - Long-acting clonidine

CRCNJ COMPLETED STUDIES

- Completed Research Studies: 24
- Epilepsy (11)
  - Levetiracetam and Cognition published recently
  - Subclinical Spikes and Neuropsychological Dysfunction
- Down Syndrome (3)
- Adolescent Schizophrenia (1)
- Adolescent Bipolar Disorder (1)
- Aggressive Behavior (1)
- ADHD (6)
- Migraine (1)

AFFILIATIONS AND COLLABORATIONS

[Logos of various collaborations]

www.theccnj.org
CNNH STAFF

www.thecnnh.org

CENTER'S RESOURCES

• Clinical Facilities
  - 5,000 square foot dedicated clinical facility
  - Two specially designed behavioral assessment/therapeutic rooms
    - Both rooms have a special rubberized and padded floor with the bottom half of the walls covered with an indestructible material ("Velcro") that can withstand various forces (such as kicking or swinging) without any damage to the wall
    - Both rooms outfitted with acoustical tiles that reduce room reverberation and avoid transmission
  - 600 square foot dedicated office for the Clinical Research Center of New Jersey, including a double-locked pharmacy room

• Laboratory Facilities
  - Dedicated laboratory room for phlebotomy, which also contains a centrifuge, and -20 and +4 degree refrigerators
  - The region's only EEG laboratory utilizing Digital Array EEG
  - Siemens System 200 (Digital Siemens, Inc.) capable of 128-channel acquisition
  - Decorated passively to allow the application of the electrodes passively and rapidly
  - Other on-site services include 16-channel intubation and cauterization without the need for sedation
  - System is capable of EEG and fMRI Potential studies

www.thecnnh.org

CNNH: CURRENT OPERATIONS

• Clinical assessment and treatment services throughout the nine county Southern New Jersey region:
  - Child Development Center (a state CFS) in Vineland
  - Obstetric Health Center in Camden
  - Linden Neuropsychiatric Stabilization Unit in Haddonfield
  - The Children's Home of Burlington County
  - Orchard Friends School
  - 50+ school districts

• Over 3,000 patient visits yearly

- ASD diagnoses (348.30 with 299.00, 299.01 or 299.80) > 500 (>150 new diagnosed)
  - ASD/OCD/R: Assessments approximately 150.
DENSE ARRAY EEG

- 128-channel Dense Array EEG is a way to electrically "image" the brain.
- The information that is gathered is placed into a powerful computer that allows the location of the electrical activity to be determined with great accuracy.
- Recent software advances in this technology now provides for the electrical source to be mapped right on to a MRI.
- Painless, noninvasive, non abrasive procedure.

www.thenchnh.org

The Philadelphia Inquirer

Legos a building block in autism therapy

Devin D'Mello

The Lego block exercises the brain and uses the Lego system represented the lego block. The lego block is a group that encourages kids to use the lego blocks for building and building skills. The lego block uses 128-channel Dense Array EEG to map the brain.

Jonathan D'Alessio, 12, of Marlborough, is one of eight researchers in a Massachusetts therapy group that uses Legos to teach social skills.
CENTER'S ENHANCEMENT PLAN: INCREASE CAPACITY

- Problem: insufficient access in NJ to quality and reliable assessment and effective intervention services for children with ASD
- Increase Capacity
  - Mobile team will travel to collaborative sites (Camden, Trenton, Theodore, and others)
  - Increase maternal/parental competence
  - Videoconferencing capabilities via mobile, web-based technologies
  - Telecommunications and behavioral assessments
  - Better continuity of care
  - Improvement in the quality of outcome data
  - Observations of low-frequency/high-intensity behaviors and severe phenomena
  - A tiered intervention in a sequential and coordinated manner
- Training and Consultation
  - Implementation of peer-based interventions to improve social, adaptive, and behavioral functioning
  - Collaboration with local service providers
  - Curriculum and life care planning integrated with objective developmental and educational assessments
  - Education and training of families, teachers and other care providers in proper intervention management and monitoring
  - Technical assistance to care providers and educational personnel and families

CENTER'S ENHANCEMENT PLAN: CLINICAL ENHANCEMENT

- Make-assessment of the trends in the literature indicates these important trends in service delivery:
  - Interventions must be tailored and done with appropriate improvements or settings
  - Individualized care and settings
  - High-impact interventions
  - High-quality interventions that ensure generalization of functional gains
  - Implementation of multidisciplinary interventions in clinical care settings, including assessment, training, and early intervention
  - Implementation of tiered, social, and emotional interventions which encompass and affect the interests and needs of children with autism spectrum disorders
  - Implementation of Tier 1, 2 and 3 levels, which emphasize typical social and emotional behavior (e.g., self-regulation, social competence, etc.)

- Health and safety continuum is a key component of the clinical enhancement process for autism spectrum services in the preschool years
  - Early identification and intervention
  - Early intervention in areas including:
    - Social cognition and social understanding
    - Social communication, include eye contact and use
    - Recreation and leisure
    - Environment and classroom
    - Educational and social skills
  - Early intervention in areas including:
    - Social cognition and social understanding
    - Social communication, include eye contact and use
    - Recreation and leisure
    - Environment and classroom
    - Educational and social skills
  - Role of the provider and agency administrators in the transition from adolescence to adulthood

www.thencnh.org
Figure 2: A 6-year-old autistic, nonverbal boy with severe aggressive behaviors that prevented a safe home environment, and whose medications necessitated the use of contingent protective tactics ("restraints"). Use of restraints (RP-420) up to 5 minutes, along with increase in the behavioral management plan, did not make an appreciable difference in the frequency and intensity of aggressive behaviors as determined by quantitative behavioral data collection (can be done via continuous interval or percent of time interval). Valley sedation (Lorazepam) was added as an alternative need stabilizer, and dosed at 10mg/day which resulted in a significant reduction in aggressive behaviors, thus eliminating the need for protective tactics. 1010, differential reinforcement of other behavior (From Wiz et al., 2009).
Autism Center of Excellence at Children’s Specialized Hospital

Yvette Janvier, MD
Uta Helme, PhD

This Center is funded in part by The NJ Children's Council for Medical Research and Treatment of Autism, Special Child Health and Early Intervention Services, NJ Dept of Health and Senior Services

Affiliated Member of the
Robert Wood Johnson Health System

Current Autism Services

Assessment
- Autism Team
- Psychology Autism Specialty
- Testing
- Neurodevelopmental
- Psychiatry
- Comprehensive Feeding Team
- Speech/Language
- Audiology
- Occupational Therapy
- Augmentative Communication
- Physical Therapy

Intervention
- Early Intervention (0-3)
- Psychotherapy
- Social Skills Groups
- Speech/Language
- Occupational Therapy
- Physical Therapy
- Sibling Programs
- Feeding Therapy
- Yoga

Current Services (continued)

Community outreach (national, state and local presentations; workshops for community & professionals)
Staff training (for Physicians on AAP toolkit; for Psychology staff on assessment, intervention; for Clinical staff on behavioral intervention)
Goals of Enhancement Grant

- CDC study shows incidence 1:150, many children diagnosed years after parents first concerned
- Need to identify and intervene early to enhance functional outcome

Clinical Expansion Program

- Developmental Screening Program
- Expanded Autism Team evaluations
- Expanded Psychology Autism Specialty evaluations
- Specialized Early Intervention services
- Expanded community outreach
- Staff training

Developmental Screening

- Provides a quick method for identifying young children at risk
- Directs children to appropriate assessment/intervention services at young ages
- Increases community awareness of early red flags
Developmental Screening Program

- Developmental Screening Clinics
  - Focus on early identification, leading to early intervention
  - 12-48 months
  - Screening by PNPs

- Pediatric Liaison Program
  - Focus on training community pediatricians to conduct developmental screening
  - Visits by PNP to staff, provide resources

The Screening Process

- Positive Screen for Autism
  - In medical setting

- Refer to Early Intervention
- Refer to Diagnostic Eval

- Determine Service Eligibility & needs
- Obtain Diagnosis & Recommendations

- Provide specialized Early Intervention Services

Expanded Autism Team Evaluations

- Offered at 3 sites (Toms River, Plaza/Mountainside, and Hamilton)
- Developmental Pediatrician, Psychologist, ST, OT
- Gold standard measures
- Assesses neurodevelopmental status, cognitive, speech/language, sensory, adaptive behavior, and family environment domains
Expanded Psychology Autism Specialty Evaluations

- Offered at all outpatient clinic locations
- Assesses adaptive behavior, cognitive skills, and autism-specific areas (ADOS, ADI-R)

Specialized Early Intervention Services

- Verbal behavior ABA model
- Establishes Autism Coordinator
- Staff training on autism screening tools (level I and 2) and ongoing consultation
- Parent workshops for additional training and support
- Establishes protocol and database to track outcomes

Community Outreach

- Type 1: Focus on increasing awareness of providers who work with young children
  Audience: daycare, preschool, DYFS
- Type 2: Parent roundtable meetings to provide education
Staff Training

- All clinical staff: behavior management training & ongoing behavioral case consultation
- Psychology staff: evidence-based ABA
- Speech/language staff: HANEN and PECS
- PNPs: autism screening tools (Level 1, 2)

Data Collection: Developmental Screening Clinic

Parent/Caregiver Questionnaires:
- Adult versions - Interactive checklist of milestones (CDC)
- Ages <16 months: CSBS; ages 16-30 months: MCHAT
- Interactive screenings:
  a. If not in EIP, administer ASQ
  b. STAT
  c. DSM IV criteria for Pervasive Dev Disorders

- Parent satisfaction survey

Data Collection: Autism Team Evaluations

Parent/Caregiver reports:
- Autism-specific: ADI-R
- Sensory: Sensory Profile
- Impact on family: Family Environment Scale
- Adaptive behavior: Vineland Adaptive Behavior Scale
- Interactive:
  a. Autism-specific: ADOS; DSM IV criteria for PDD
  b. Cognitive: DAS II or Mullen
  c. Speech/language: 2-3 yrs-REEL; over 3-CASL
  d. Occupational: 2-6-Peabody Dev Motor; 2-up;
- Parent Satisfaction survey
Data Collection: Psychology Autism Specialty Evaluations

- Parent/Caregiver reports:
  - autism-specific: ADI-R
  - adaptive behavior: Vineland Adaptive Behavior Scale
- Interactive:
  - autism-specific: ADOS
  - cognitive: DAS II or Mullen
  - clinical interview; DSM-IV Pervasive Developmental disorders review
  - Parent Satisfaction survey

Evaluation of Clinical Enhancement Program

Goal: Identify and provide intervention at earlier ages
1. Compare # and ages of new patients diagnosed with ASD through Developmental Screening Clinics, Team and Psychology Autism Evaluations versus # and average age of new patients seen at baseline
2. Compare average wait from referral, evaluation, & inception of EIP versus baseline
3. Assess consumer satisfaction with Autism Team & Developmental Screening evaluations, Pediatric liaison service and outreach workshops

Timeline Estimates

- Developmental Screening: July 2008 for screening appoints; Pediatric liaison visits to start by 8/28
- Autism Team Evaluation 1 slot/week @ each of 3 sites: July 2008
- Autism Team Evaluation 2nd weekly slot: Jan 2008
- Psychology Autism Specialty Evaluation: July 2008
- EIP Autism Coordinator: July 2008
- EIP Staff training: October 2008
- EIP Parent training workshops: April 2009
- Pay staff training: step-wise thru March 2009
- Clinical staff behavioral training: July 2008, ongoing
- PNP Level I and Level II Autism tool training: July 2008
- Parent Roundtable workshops: December 2008

Community workshops for DYFS, daycare, preschool: April 2009
New Jersey Governor's Council on Autism
Clinical Enhancement Program

Institute for Child Development
The Joseph M. Sanzari Children's Hospital
Hackensack University Medical Center

Randye F. Huron, M.D.
June 24, 2008

Goals of Enhancement Program

- Improve access to services
- Increase scope of intervention for ASD children and families
- Increase community outreach
  (conference, website)

Institute for Child Development

- Provides multi-disciplinary evaluation and treatment for infants, children and adolescents with developmental and behavioral disorders
- System of evaluation and therapy is individualized based on child's and family's needs
- Extensive education commitment and community outreach

I.C.D. 2007

- 32 FT, 10 PT professionals and para-professionals
- Developmental and behavioral pediatricians
- Nurses
- Child and adolescent psychiatrist
- Special educators (LDT-C)
- Social workers
- Psychologists
- Speech-language pathologists
- Occupational therapists
- Physical therapists
- Audiologist
- Independent child study team

I.C.D. 2008

- 30 FT, 3 PT professionals and para-professionals
- Developmental and behavioral pediatricians
- Nurse
- Speech and language pathologists
- Occupational Therapists
- Physical Therapists
- Audiologists

GRANT POSITIONS

- P/T Program Coordinator
- P/T Developmental Behavioral Pediatrician
- P/T Social Worker
- P/T Early Childhood Specialist/LDT-C
- FT speech/language pathologist
- 1.5 FTE support staff
**Goals: Improve access to services/increase scope of services**

- Dedicated intake coordinator services/functions
- Conducting additional DPS evaluations
- Expanding social skills groups
- Expanding parent education/support
- Increasing availability speech/language therapy

- Expanding medication management
- Coordinated interdisciplinary ICD “team” (to plan for groups, case coordination, journal club, plan conferences, etc.)
- Interdisciplinary Children’s Hospital pediatric team (developmental pediatricians, child and adolescent psychiatrist, nurse, audio, geneticist, pediatric GI, pediatric pulmonary (sleep), pediatric neurologist

**Goals: Improve access to services/increase scope of services**

- Parent meetings scheduled after diagnosis for assistance and support between diagnosis and therapy (Social work, learning consultant or developmental pediatrician)
- Parent training and education program (Alpine Learning Group providing 8 in-home parent training program) for newly diagnosed.

**Parent Training and Education Program**

- Saturday training – 8 hours – open to extended family and caretakers
- 8 hour individual in-home parent training sessions

**Newly Diagnosed Family**

- Provide clear information about available resources and services
- Prioritize recommendations
- Assist parents in making decisions about child’s treatment, intensity, frequency, location
- Provide support and information (websites, respite, sibling support)

**Improve Community Educational Outreach**

- Host conferences re: autism
- Public Relations – brochure, website with links to other important programs/services
Who makes the diagnosis?

5 Developmental and Behavioral Pediatricians

Instruments you typically use for a given aged patient:

0-3 years  Capute Scales
3-6 years  Lexington Developmental Scales
6 and up  General Information Questions
          Analogy Questions
          Vocabulary
          Digit Span
          Sequential commands
          Draw-a-person
          Slosson Drawing Coordination Test
          Wide Range Achievement Test

Data Collected

- Presenting problems/Concerns
- Birth history
- Developmental history
- Past medical history
- Social history
- Early Intervention/Pre-K/school history/information
- Interventions
- Family history
- Autism DSM IV criteria

How the data are being filed and housed

- In patient charts in chart room
- Will create separate cost center for grant
- Will create separate activity codes for each grant supported service
- Revenue and usage reports
- Daily log of activity
- Next available appointment monitoring
Hunterdon Medical Center
Child Development Center

Hunterdon Medical Center's
Child Development Center
30 year history of providing multidisciplinary
 diagnostic and therapeutic services
• Developmental Pediatric Associates
• Pediatric Rehabilitation
• Independent Child Study Team
• Multidisciplinary evaluations
• Special Child Health Services
• Early Intervention Program
• Speech and Hearing Department
• Hunterdon Behavioral Health

Key Program Staff
Carol M. Klein, MA-CCC-SLP, MPA
Administrative Director Rehabilitation Services
Developmental Pediatric Associates:
Audrey Marx, MD, Medical Director
David Atkin, MD
Francis Urofsky, MD
Michael Williams, MD
Lori Kennedy, FNP
Cynthia Oser, FNP
Special Child Health & Early Intervention Services:
Susan Freedman, CCSP, Special Child Health Services
Linda O'Brien, RN, MSN, Early Intervention
Virginia Hope, RN, BSN, Early Intervention
Current Issues

- Wealth of patient information may be better organized
- Need for more cohesive care within the community
- Need for more access to social skills/home based behavioral programs
- Increased demand on limited resources for transition planning as children become young adults

The Hunterdon Medical Center
Regional Autism Center

Enhancement of clinical services for the Autism community of Hunterdon, Somerset and Warren Counties
Will see Mercer as needed
Focus of Grant: Community Access

- Outreach to schools and community providers to improve outcomes throughout the lifespan of individuals affected by autism spectrum disorders
  - Meet with local family practitioners
  - Meet with representatives from community providers
  - ABC CEO Tri-County CAG
  - Develop area resource guide for transition services
  - Establish lending library with transition-related books
  - Implementation summer 2008
  - Provide training for health & educational professionals
    - Fall 2008
Focus of Grant: Clinical Coordination

• Outreach to schools and community providers to improve outcomes throughout the lifespan of individuals affected by autism spectrum disorders
  – Hire social worker/ Autism center coordinator of services
  – Liaison between families, community and service providers
  – Summer / Fall 2008

Focus of Grant: Access to Intervention

• Increased access to behavioral and pharmacological treatment paradigms
  – Implement home-based behavioral programs for children with ASD
  – On-site social skills training programs
  – Case conferences with psychiatry to more effectively manage co-morbidities
  – Commence summer / fall 2008

Focus of Grant: Coordination & Collaboration

• Increase access to multidisciplinary assessment and treatment programs that address the broad symptomatology of ASD
  – Utilize social worker to facilitate and engage children who will benefit from multidisciplinary evaluation and referral for therapies on site
  – Introduce staff to clinical use of ADI-R, ADOS-G
  – Hire data manager to input relevant patient data
  – Demographics, diagnostic information, co-morbid conditions, treatment plan, interventions, and post-intervention
  – Fall / winter 2008
Special Programs

Library will include:
Multi Media Materials for Professionals & Families including:
  DVD's for group presentations
  Applications for programs such as DDD
  Materials specific to transition to adult life

Special Programs

Technical Support for Professionals

Warm Line

Developmental Pediatrician will provide a warm line 2 hours a week for direct contact between health and educational professionals for case discussion and patient referrals

Major Service Enhancements

<table>
<thead>
<tr>
<th>Type of Service</th>
<th>Current Local Level of Services</th>
<th>Possible Level of Services</th>
<th>Number of Additional Services</th>
<th>% Increase Additional Services</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montessori</td>
<td>120</td>
<td>175</td>
<td>55</td>
<td>19%</td>
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<td>ABA</td>
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<td>50%</td>
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<td>Speech Language Pathology</td>
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<td>160</td>
<td>100</td>
<td>17%</td>
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<td>10</td>
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<td>50%</td>
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<tr>
<td>Total Developmental Disabilities</td>
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<tr>
<td>Mental Health Evaluation</td>
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<td>Mental Health Evaluation</td>
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<td>Repetitive Behaviors</td>
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<tr>
<td>Repetitive Behaviors</td>
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<tr>
<td>Behavioral Characteristics</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Data Collection: Demographics

Demographics to include:
- County/zip code
- Referral source
- Age of birth
- Parent's educational history/occupation
- Parent's ages at conception
- Type of Health Insurance
- Family ethnicity
- Family history
  - Multiplex or simplex family
  - Siblings (developmental delay?)

Data Collection: Clinical

- Diagnosis based on DSMIV-TR
- Cranial diagnosis
- Age of initial diagnosis, history of regression
- Relevant past and Medical history
- Feeding physical findings
  - Feeding, Gastroesophageal reflux
- Treatment Plan
  - Behavioral therapy
  - Medical therapy
- Interventions
  - Pharmacological therapy
  - Submitted to speech therapy
- Test results
  - SRT: 6
  - LSAT
  - ATIS
  - SALT:
- Developmental levels at initial evaluation
- Language level expressive/receptive
- PPVT: Expressive vocabulary testing
- VOTCAR: Neurodevelopmental test

Data Collection: Statewide Network
Components of the Program

- Infant Toddler Assessment Program
- Child Evaluation Center
- Child Behavioral Health

Infant Toddler Assessment Program (ITAP)

- Monitoring program for high risk infants and toddlers
  - Babies born prematurely
  - Term babies with difficulty in neonatal period
  - Babies with prenatal exposure to drugs and alcohol
  - Children suspected of developmental delays
- Birth to 3 years
- Secondary screening for children with features suggestive of ASD
Infant Toddler Assessment Program (ITAP)

- Children seen every three months (corrected for prematurity) until 18 months then every 6 mos.
- Team screening with OT/PT, speech therapist, psychologist, nurse and developmental pediatrician
- Team meeting about each child at the end of each session
- Note sent to primary physician with recommendations

Child Evaluation Center

- Referrals for developmental assessment
- 2-21 years
- Team evaluations may include: psychological, educational consult, speech and language evaluation, occupational therapy evaluation
- One of the 11 state funded CECs

Child Evaluation Center

- Team includes
  - 2 developmental pediatricians
  - Psychologist
  - Learning consultant
  - Social worker
  - Nurse
**Child Evaluation Center**
- Pediatric neurologist sees patients one half day/week
- Fetal Alcohol Diagnostic Center—one of 6 state funded FAS centers
- Speech and language therapists and occupational therapists part of Progression Rehab. Available for consultation

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**Child Evaluation Center**
- Current care for children suspected of autism spectrum disorder includes:
  - Developmental assessment
  - Structured interview with GARS/GADS
  - Consideration of ADOS
  - Cognitive assessment
  - Diagnosis, recommendation and physician developmental monitoring

---

**Behavioral Health at JSUMC**
- Therapeutic program provides group socialization/play therapy for 3-6yr.
- Parenting component while children participate in group
- Option to see the child psychiatrist
- 4 child psychiatrists part of behavioral health
Monmouth County Early Autism Center

- Key feature-center wide medical home concept
- System building and community development
- Family focused individualized collaborative care management
- Goal is maximizing child’s developmental potential for optimal functional outcomes.

MEAC-Objective 1: Promote early identification of Autism

- Increase the organized screening and early identification of children between 1-6yrs. with signs and symptoms of ASD
  - Office based practice change methods to increase understanding and earlier identification of autism
  - Collaboration with Pediatric Council On Research and Education /AAP for office based training to primary physician for earlier identification of high risk children

MEAC-outcomes measures

- Early identification: PCORE to develop practice based module on early id of autism
- 8 practice sites the first year
- Additional 20 practice sites 2nd year
- Goal is increase screening with M-CHAT in primary care to 80% in Monmouth County
MEAC-Objective 2: Secondary Screening

- Develop and implement accessible and rapid process for secondary screening of children identified by primary care in order to triage needs for comprehensive assessment and delivery of services.
- ITAP: Provide secondary screening for children who fail M-CHAT screening at well child visit.

MEAC-Objective 2: Secondary Screening at ITAP

- Provide family with resources to access intervention services quickly
- Monitoring of child's progress
- Seamless referral for full evaluation
- Grant will allow for hiring of a nurse practitioner to increase capacity for case management and secondary screening

MEAC-outcomes measures

- Secondary screening in ITAP
- ITAP to extend hours to allow for up to 35 children a month to be screened
- Child seen within 3 months of primary care visit
- Referral for initiation of services before full diagnosis
- Family support services and care management
MEAC Objective 3: Comprehensive assessment

- Provide timely comprehensive assessment, diagnostic and care management 'medical home' services
- Psychologist to increase time for diagnostic services
- Behavioral specialist to consult on services
- Project coordinator to oversee administration of grant

MEAC-outcomes measures

- Team assessment and diagnosis
- CEC to have capacity to add up to 20 children per month for assessment of ASDs

MEAC Diagnostic Process
MEAC Objective 4:
Expand and coordinate services
- Expand, enhance and coordinate the number and depth of language, social skills and behavioral therapy for young children with ASD.
- Increase collaboration with Behavioral Health and Progressions Rehab.
- Grant allows for speech and language therapist as part of CEC team

Monmouth County Early Autism Center-behavioral health
- Hire behavioral specialist in autism to oversee therapeutic interventions
- Case manage
- Collaborative team meetings
- Establish therapeutic groups specifically designed for young children with autism: social skills and behavioral intervention

MEAC Objective 5: Collaborate with community agencies
- Increase community awareness of autism and increase support services for families and professionals
- Monmouth Cares: county care management organization for behavioral health
  - Web-based network for increasing awareness and providing community resources
- Division of Developmental Disabilities
MEAC Objective 5: Collaborate with community agencies
- Collaborate with Parent support organizations
  - POAC- educational trainings for families and professionals
  - CoSAC

Monmouth County Early Autism Center-Data collection
- Establish database with Access
- Define variables to measure
- Define outcomes measures

Tracking performance indicators
- Goal #1: earlier identification
  - # of children referred by primary care
  - # of Primary care providers using M-CHAT
- Goal #2: secondary screening
  - # of secondary screens conducted
  - Average wait in days from secondary screen to full evaluation
  - # given recommendation and referrals for intervention
  - # referred for full eval and % of those screened
Tracking performance indicators

- Goal #3: Diagnostic assessment
  - # of comprehensive assessments completed
  - Wait time to diagnosis
  - # of children diagnosed (with demographics)

- Goal #4: Expand/coordinate services
  - # and % of children admitted to therapeutic program
  - # of children receiving speech/lanq. services
  - # of children with individualized treatment plan

Tracking performance indicators

- Goal #5: Community awareness/support
  - # of public forums
  - % satisfaction with public forums
  - # of cultural and language appropriate support services offered
  - % family satisfaction with support services

Outcomes

- Monitoring process
- Measurement of child's improvement in language, social and behaviors
- Family and primary provider satisfaction with care and feedback provided
- Ongoing team approach to patient care
The Autism Center of New Jersey Medical School is a coalition of researchers, clinicians, parents, educators, and service providers who are committed to eliminating autism spectrum disorders in current and future generations and improving the quality of life for the growing number of persons affected by ASD.

TAC’s Multidisciplinary Team emphasizes
- Early detection
- Delineation of medical and functional status
- Individualized treatment
- Utilization of effective medical, educational and behavioral interventions.

Governor’s Council Grant Priorities
- Early Identification- through Fast Track
- Collaboration with existing resources
- Multidisciplinary Care Enhanced
- Standardized Diagnostic Assessment-
  - ADOS/Cognitive/Speech and Language/OT
- Community Outreach/medical education
Early Identification

Fast Track - through comprehensive Child Evaluation Center (Team will be lead by Child Psychologist)
- Reduce delays in identification and diagnosis of our patient population.
- Directed at patients less than 5 years of age who have not received a diagnosis and are suspected of an ASD.
- To specifically address the latency of diagnosis within the greater Newark Region (Mandell et al. 2006), patients age 7 and younger who have not received formal diagnosis will be included in our Fast Track system.

Early Identification
Fast Track cont.
- The Fast Track program involves identification of patients based upon age and geographic indicators
  - Streamlined intake system - with collaboration with the CEC
    - Create phone interview to be conducted by nurse in the CEC
    - Phone interview with MCHAT completed
    - Translation services to facilitate the intake process
    - Referral to and from EI/ or other centers
    - Direct collaboration with PCP
    - Clinician time in the Child Evaluation Center
  - Clinical time will be reserved for fast track screen
  - Scheduling delays will be monitored

Early Identification
Outreach

Community/ allied health outreach
- Improve ID rates and enhance intervention and treatment outcomes
  - Community and site based training for health care providers
  - Introduction of the AAP guidelines
- Focus groups for the new ID Toolkit
  - A.S.K. Autism support kit
    - Information on what to expect within the center
    - Information about ASD and medical co-morbidities
    - Resource guide with storage capacity as well as numerous downloadable forms and sample letters for families.
    - All information provided in the resource kit will also be available on CD for easy access and retrieval.

Enhanced Multidisciplinary Team Model

Team building
- Increase capacity
- Reducing wait times
- Facilitating early identification target
  - Area of the greater Newark Region
- Enhancing diagnostic and clinical care

Multidisciplinary Care
Enhanced Clinical Model

As illustrated above, the CEC nurse is responsible for triage during the initial intake process. The nurse coordinator sets referral of the patient to Individual Specialists, the Biomedical Assessment and Treatment Track, Brain & Behavior Track. A nurse coordinator also facilitates any clinical diagnostics by assisting in the administration of behavioral, quality of care and quality of life instruments. (See the Diagnostic Assessment matrix)
Enhanced Clinical Training for all new hires to TAC
- Proved a forum of case conference
- Direct observation
- Small group trainings

Standardization Across Domains
- Diagnostic Assessment
- Outcome Monitoring
- Equity of service
- Satisfaction of Service

Diagnostic Assessment Tools
Tools – including streamlined intake
- Diagnostic - Initial
  - ADOS-G, Autism Diagnostic Observation Schedule-General (Clinician and Child)
  - GARS, Gilliam Autism Rating Scale (Clinician)
  - DSM-IV Symptom Checklist (Clinician)/CBCL
- Medical/History
  - Vital Signs, Includes Height, body mass index, abdominal circumference, etc (Nurse)
  - Physical Exam w/ Pertinent Review of Medical Hx, + dysmorphic head circumference (Clinician)
- Cognitive
  - TONI-3, Test of Nonverbal Intelligence, for non-verbal (Clinician) or
  - DAS II, Differential, for verbal (Clinician)
- Language- ROWPVT/RowPVT or CASL
- Psychosocial- PedsQL/PSI

Diagnostic Assessment Tools
Tools
- Brain and Behavior
  - Comprehensive intake/ neurological exam
  - Vital signs* enhanced (BMI, AC)
  - DSMIV initial
  - GARS initial and q 6 months
  - CBCL- initial and every visit
  - PedsQL/PSI q 6 months
  - ABC and CGI q 6 months

Diagnostic Assessment Tools
Tools
- Biomedical
  - Comprehensive intake/neurological exam
  - Physical and enhanced physical
  - CBCL each visit
  - Sensory profile q 6 months
  - GARS every six months
  - ABC every six months
  - PedsQL/PSI q 6 months

Outcome Monitoring
Tools
- ABC, Aberrant Behavior Checklist (Nurse and Parent)
- CGI, Clinical Global Impression Scale (Clinician)
Equity of Service

Tools
- PedsQL, Pediatric Quality of Life, 4 versions, based on AGE (Nurse and Parent)

Satisfaction of Service

Tools
- Multidimensional Assessment of Parental Satisfaction (MAPS) for Children With Special Needs (Ireys et al. 1999)
- Assessment of Chronic Illness Care (PACIC) (Glasgow et al. 2005)

This clinical enhancement proposes a model for biomedical care that aims to improve the quality, access to care, and outcomes for patients affected by ASD in the underserved community of the greater Newark region. This model is the result of evidence based medicine as well as our collective clinical experience as a diagnostic and treatment center.
Clinical Database Development
NJ Governor's Council on Autism, Clinical Enhancement Program
June 24, 2008

Project Planning

- Needs Assessment
  - Program Specific Objectives
  - Center Specific Objectives
- Resource Assessment
  - Clinical Systems
- Standards Assessment
  - Information Standards
  - Data Transmission Protocols
- Technology Assessment
  - Database Standards
  - Application Standards
  - Access & Publishing Protocols
- HIPAA Considerations
  - Access Control, Incident Tracking, Multi-Tiered Privileges and Granting, etc.
Program Objectives

- Medical/Family History (using the NJ-ACC standardized form to be provided by the New Jersey Governor’s Council on Autism after the award)

- Physical/Neurological examination, performed by trained physician or nurse practitioner (using the NJ-ACC standardized form to be provided by the New Jersey Governor’s Council on Autism after the award)

- Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS), used according to their published manuals, Vineland Adaptive Behavior Scales, Second Edition, to be administered upon confirmation of DSM-IV diagnosis of ASD.

- An IQ or developmental assessment, that includes both nonverbal and verbal components and results in standardized scores for both.

Needs Assessment

Program Objectives

- Patient Info
- Clinical Assessments
- Patient Status
- Project Reports

Center Objectives

- Insurance Info
- Referring Physicians
- Medications
- Diagnosis & Procedures
- Patient Reports
- Patient Requests
- Reports, Letters & Others
- Prescriptions
- Prescription Management
- Document Management
- Patient Scheduling
- Adverse Events
- Lab Results
- Medical Training
- Do the current resources meet the goals of the project or Center?
- To what extent can the database be integrated with existing systems?
- How can we avoid double entry of data?
**Standards Assessment**

**Information Standards**
- SNOMED-CT
- ICD-9
- CPT
- RxNorm
- CTCAE

**Data Exchange Standards**
- Information Standards
  - Database Standards
    - Patient Scheduling
    - Interoperability
- Data Exchange Standards
  - Lab Results

**NLM-supported standards for terminology, ontologies and frameworks, that are maintained by The National Library of Medicine (NLM).**

- **Medical Coding:** SNOMED CT (Systematized Nomenclature of Medicine - Clinical Terms)
  - Mappings between clinical vocabularies (SNOMED CT to ICD-9-CM, SNOMED CT to CPT)
- **Prescription Data:** RxNorm provides standard names for clinical drugs:
  - active ingredient + strength + dose
- **Laboratory Data:** LOINC (Logical Observations Identifiers, Names, Codes)
  - Mappings between clinical vocabularies (LOINC to CPT)
- **Adverse Event Reporting:** CTCAE (Common Terminology Criteria for Adverse Events)
  - CTCAE is designed as a clinical trials research tool

---

**Technology Assessment**

**Database Standards**
- Microsoft SQL
- Microsoft Access
- Oracle
- FileMaker
- MySQL

**Application Standards**
- Microsoft Access
- Web Application
- PHP
- FileMaker
- ASP.NET

**Access**
- Direct Application
- Application Publishing
- Citrix
- Terminal Server
HIPAA Considerations

1. **Audit Log**: Tracking key events: Login/logout, new/delete patient, access to each patient, new contacts, etc. Current system provides audit log for key events/fields.

2. **Incident Tracking**: Failed login reporting and attempts to login.

3. **Access Control**: Login procedure is three levels; 1) Network 2) Citrix and 3) Access/SQL form based w/ DB encryption.

4. **Multi-Tiered Privileges and Granting**: 1) View; 2) edit & view, no delete; 3) Add, edit, view; 4) Delete, Add, View, Edit; 5) Delete, View, Edit, Add, Admin (passwords, users, questions, reports, exporting data).

5. **Contingency Planning**: Super-Admin login and regular backups.

6. **User Documentation**: Full user documentation will be provided by developer.

7. **Auto Log-off**: Network, Citrix and Access based.

8. **Encryption**: Local encryption and network encryption through Citrix and VPN.

9. **Data Integrity**: Access to database is exclusively through a Citrix encrypted channel.

10. **Data Validity**: Quarterly review of data acquisition with column list reports and review of data formatting and column contents.

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Project Overview

![Diagram of project overview]

**Core Functions**
- Patient Info
- Clinical Assessments
- Project Reports
- Document Management
- Adverse Events
- Lab Results
- Medical Training
- Prescription Management
- Patient Request System
- Patient Reports
- Patient Scheduling

**Optional Functions**
- Collaborative Functions
Technical Contributors

Paul J Zumoff, Ph.D. — Microsoft Access/SQL Development
Database Developer

Alexandre Peshansky, M.S. — Information Standards
Informatics Core Director
Clinical Research Center-UMDNJ
APPENDIX D

- Information page on how to use the Birth Defects Registry / Governor’s Council on Autism Registration Software
- Web shots of the fields included in the database
January 29, 2009

Introduction

The BDR data entry software has been developed for use by facilities and providers registering individuals in NJ to the state Birth Defects Registry. The main function is to provide an electronic version of the SCH-0 Special Child Health Registration form, and the Autism Supplemental form. A section of the software is provided for grantees of the Governor’s Council to record further detailed registrant information for local research, and for consolidation into a state-wide database of family, medical, and treatment history managed by the NJ Department of Health.

The application consists of 8 forms, with some forms and sections optional, depending on whether a state registration is being created, and whether an Autism diagnosis is included. A Global User Id (GUID) is created for all registrants to maintain requested parental anonymity and for Grantee participants whose data is reported to the state but remain unregistered to the State BDR registry.

Fig. 1 – Form navigation command buttons.

Using the Forms

The application opens to the initial Child Registration form which is the hub of navigating the remaining forms. A new entry can be created by immediately typing into the form fields. When the page is as complete as possible, click on the “Error Check” button at the top of the page to check that required fields have been entered properly. Any omissions or errors will be highlighted in blue for correction. When no errors have been found, a message will display to that effect and the command buttons (Fig. 1) for the other forms will be enabled.

The ChildReg form must be completed and Error Check’d before the other forms can be accessed. In order to complete a registration for submission to the State, the first 4 forms must be completed. The forms can be printed for submission as a registration by clicking the printer icon button on the row of command buttons.

Once a specific form has been Error Check’d, no changes can be made to form unless the “Edit Unlock” button is clicked. This will prevent accidental changes to validated data.
The forms required for a basic registration to the State are “Child Reg”, “Parent Guardian”, “Diagnosis History”, and “Tests and References”, from left to right on the command buttons.

![Microsoft Access - [Evaluation History]](image)

**Fig. 2 –** Grantee Detail/Followup Section navigation command buttons.

The Grantee Detail forms, in its own section, is illustrated above (**Fig. 2**).

To access a previously entered registrant, select and click on a name from the drop down box (“Select a name…”) at the top left of the Child Registration form.

Remember to “Error Check” a form whenever data is entered or changed. This will ensure the data is checked for correctness, and locks the page from further unintended changes.

Attached/enclosed are screen prints of example forms completed to date. “Med Detail” and “Follow Up” are currently in development. The anticipated availability of the complete application is for March, 2009.

Thank you for your time and consideration in reviewing these documents.

NJ Dept. of Health and Senior Services  
Early Intervention and Monitoring Program  

The Governor's Council for Medical Research and Treatment of Autism  
[http://www.state.nj.us/health/autism/index.shtml](http://www.state.nj.us/health/autism/index.shtml)
WEB SHOTS OF THE FIELDS INCLUDED IN THE COLLECTIVE DATABASE
Grantee Detail / Followup Section  
NJ DOHSS - BDR Registration

A-V-C Test Results

Process Current Form
Click to process current section

Error Check  Edit Unlock  Undo  Quit

Only ONE of the tests may be entered per page. To enter additional test, Click for New Record.

Test Date  
2/1/2009 - Autism Diagnostic Observation Schedules  
2/3/2009 - Vineland Adaptive Behavior Scales

Instruments/Reference

Test Result Date
2/1/2009

Is this the first administration of the test entered below?
○ First Time  ○ Re-Assessment

Entry Directions
New Record

A. To enter First test result, begin typing.  
B. For additional new tests, Click New Record button and click the icon:  on Nav bar below

ADOS Results

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<thead>
<tr>
<th>Communication (0 - 10)</th>
<th>Reciprocal Social Interaction (0 - 14)</th>
<th>Imagination/Creativity (0 - 4)</th>
<th>Stereotyped Behaviors Restricted Behavior (0-8)</th>
<th>ADOS Addl Notes</th>
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</tbody>
</table>

Vineland (VABS-II) Results

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<tr>
<th>Standard Scores (20 - 160)</th>
<th>Percentile Scores (0 - 14)</th>
<th>Vineland Addl Notes</th>
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<tr>
<td>Communication</td>
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<td></td>
</tr>
<tr>
<td>Daily Living</td>
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<td>Socialization</td>
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</table>

CELF Results

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<th>CELF Addl Notes</th>
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<td>Language Content</td>
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</tr>
<tr>
<td>Language Structure</td>
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</tr>
<tr>
<td>Working Memory</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

End A-V-C Page
**Evaluation History**

**Guidance**

- **Guideline**: The form is for recording evaluation history. It is used to track the progression of a child's development and any relevant medical history. The form includes sections for recording the date of evaluation, the child's current and previous diagnosis, referrals, and the types of providers involved.

- **Instructions**: The form is designed to be filled out by healthcare professionals, typically during routine check-ups or when a child is being evaluated for a new condition. It is important to record all relevant information accurately and in a timely manner for effective patient care.

**Columns**

<table>
<thead>
<tr>
<th>Current and Previous Diagnosis(es)</th>
<th>Current</th>
<th>Previous</th>
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<tbody>
<tr>
<td>Autistic Disorder</td>
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<tr>
<td>Autism Spectrum Disorder</td>
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<tr>
<td>Asperger's Disorder</td>
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<td>PDO-NOS</td>
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<tr>
<td>CDD</td>
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<td>Rett Syndrome</td>
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<tr>
<td>ADHD</td>
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<td>Fragile X</td>
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<tr>
<td>Epilepsy/Seizure disorder</td>
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<td>Mitochondrial disease</td>
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<tr>
<td>Cerebral Palsy</td>
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<td>Down's Syndrome</td>
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<tr>
<td>Bipolar Disorder</td>
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<td>Fetal Alcohol Syndrome</td>
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<td>Tubercerous Sclerosis</td>
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<tr>
<td>Neuromuscular disorder</td>
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<tr>
<td>Depression</td>
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<tr>
<td>Anxiety disorder</td>
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<td>Mental Retardation/Intellectual Disorder</td>
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<td>Sleep disorder, Insomnia, Narcolepsy, Other</td>
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<td>Irritable bowel syndrome</td>
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<td>Other Gastrointestinal disorders</td>
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<tr>
<td>Speech/Language Delay</td>
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<td>Developmental Delay</td>
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<td>Learning Disability</td>
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<tr>
<td>Repetitive/Stereotyped Behavior/Activities</td>
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</tbody>
</table>

**Referrals**

- **Who referred (Check all that apply)**
  - School
  - Pediatrician
  - Family Physician
  - Psychologist
  - Psychiatrist
  - Family referral
  - Community-based agency
  - Other, please specify

- **Where referred (Check all that apply)**
  - Speech Pathologist
  - Occupational therapist
  - Physical therapist
  - Developmental Psychologist
  - Educational program
  - Behavioral program/social skills
  - Home program
  - Other, please specify
APPENDIX E

- The web shot of the front page of the Governor's Council's website
WEB SHOT OF THE FRONT PAGE OF THE GOVERNOR'S COUNCIL WEBSITE
The Governor's Council for Medical Research and Treatment of Autism

Overview and Mission

The mission of the Governor's Council is to establish a Center of Excellence in the State where basic science and clinical research studies, and clinical diagnosis and treatment initiatives can take place. To this end, the Council awards grants and contracts to public and private nonprofit entities.

The Governor's Council for Medical Research and Treatment of Autism was formed by statute in 1999 and was situated at the University of Medicine and Dentistry of New Jersey (UMDNJ), until September 12, 2007 when Governor Corzine signed into law a measure to move the Council to the New Jersey Department of Health and Senior Services (NJDHSS). The Council's initiatives are funded by a one dollar surcharge from motor vehicle violations and fines, which results in approximately $4 million being dedicated to autism research, treatment and education annually.

In moving the Council from UMDNJ to the NJDHSS, Governor Corzine also expanded the Council's membership from a six-member to a fourteen-member board. The Council's membership is made up of representatives from academic institutions, autism and healthcare organizations, appointees of the Senate President, Assembly Speaker and Commissioner of Health, and also includes a member from the general public, and an individual with autism, or family member.

What is Autism?
This link from the Centers for Disease Control and Prevention (CDC), lists Frequently Asked Questions (FAQs) about Autism.