Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Children's Hospital of Philadelphia (CHOP)

2. **Reporting Period (start and end date of grant award period):** 6/1/2009-5/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Nicole M. Young, B.S.

4. **Grant Contact Person's Telephone Number:** 267-426-7747

5. **Grant SAP Number:** 4100047863

6. **Project Number and Title of Research Project:** CHOP/Penn Center of Excellence for Autism Research

7. **Start and End Date of Research Project:** 6/1/2009-5/31/2013

8. **Name of Principal Investigator for the Research Project:** Robert Schultz, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $4,723,288.85 ($782,649.97- indirect costs)

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	Institution	% of Effort on Project	Cost
Abel, Edwin (Ted)	Co-PI - Project 3	Penn	Year 2 - 5%; Year 3 - 2%; Year 4 - 3%	$27,296.87
Abrams, Debra	Data Analyst - Project 1	CHOP	Year 4 - 10%	$10,657.03
Ashtari, Manzar	Co-PI - Project 5	CHOP	Year 1 - 10%	$22,409.88
Baguio, Fottfam	Behavioral Research Assistant - Project 4 & 5	CHOP	Year 4 - 50%	$23,208.43
Bartley, Gregory K.	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 4 - 17.5%	$7,117.50
Bradstreet, Lauren	Behavioral Research Assistant - Project IV & 5	CHOP	Year 2 - 100%; Year 3 - 100%; Year 4 - 8.25%	$70,425.23
Brodkin, Edward (Ted)	PI - Project 3	Penn	Year 1 - 4%; Year 2 - 4%; Year 3 - 10%; Year 4 - 8%	$62,528.92
Browne, Aaron	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 2 - 25%; Year 3 - 47%; Year 4 - 29.5%	$45,648.08
Bucan, Maja	PI - Project 2	Penn	Year 1 - 10% Year 2 - 10% Year 3 - 10% Year 4 - 12.6%	$56,228.38
Bush, Jennifer	Behavioral Research Assistant - Project 4 & 5	CHOP	Year 2 - 12.5%; Year 3 - 43.75%; Year 4 - 6.25%	$21,152.45
Cannon, Katelyn	MEG Research Assistant - Project 5	CHOP	Year 2 - 22%; Year 3 - 33%	$14,478.67
Caramanico, Julie	Clinician - Project 4	CHOP	Year 2 - 37.5%; Year 3 - 43.75%; Year 4 - 31.25%	$60,289.77
Carolan-Tomkinson, Megan	Clinician - Project 4	CHOP	Year 3 - 8.75%	$7,027.85
Chiavacci, Rosetta	Genetics Program Director - Project 1	CHOP	Year 4 - 10%	$9,803.68
Chikkagoudar, Satish	Post-doc - Project 2	Penn	Year 2 - 13.5%	$6,742
Connell, James	PI – Project 6	Penn	Year 2 - 12%; Year 3 - 15%; Year 4 - 5%	$18,113.57

Davatzikos, Christos	PI – Project 5	Penn	Year 2 - 5%; Year 4 - 2%	$18,174.00
DeLussey, Christine	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 3 - 81.25%; Year 4 - 26.25%	$42,288.46
Doehring, Peter	PI - Project 6	CHOP	Year 1 - 2.5%; Year 2 - 5.75%	$14,756.42
Dow, Holly	Research Assistant - Project 3	Penn	Year 3 - 50%; Year 4 - 25%	$16,979.69
Dunn, Debra	Recruitment Director - Project 4	CHOP	Year 2 - 6.25%; Year 3 - 21.25%; Year 4 - <1%	$26,262.96
Eavani, Harini	Biomedical Engineering Graduate Student - Project 5	CHOP	Year 2 - 60%; Year 3 - 5%	$34,278.65
Edgar, James C.	Co-Investigator - Project 5	CHOP	Years 1-4 - 5%	$31,597.52
Epstein, Susan	Clinician - Project 4	CHOP	Year 1 - 33%; Year 2 - 33%; Year 3 - 24.75%	$111,951.67
Filipovych, Roman	Post-doc - Project 5	Penn	Year 1 - 22%; Year 2 - 50% Year 3 - 30%	$62,862.00
Flory, James	Research Assistant - Project 1	CHOP	Year 1 - 20%	$8,156.89
Fretz, Julianne	Recruitment Assistant - Project 4	CHOP	Year 2 - 12.5%; Year 3 - 42.5%; Year 4 - 2%	$21,767.92
Gabrielsen, Terisa	Post-doc - Project 4	CHOP	Year 4 - 5%	$2,533.60
Georgi, Benjamin	Post-doc - Project 2	Penn	Year 2 - 16.7% Year 3 - 33.3%	$22,980
Giovannelli, Daniel	Summer Undergrad - Project 5	CHOP	N/A – Hourly paid	$1,065.93
Giserman, Ivy	Behavioral Research Assistant - Project 4 & 5	CHOP	Year 4 - 8.25%	$1,600.30
Golhar, Abhay	Bioinformatics Specialist - Project 1	CHOP	Year 4 - 25%	$56, 495.31
Hakonarson, Hakon	PI - Project 1	CHOP	Years 1-4 - 1%	$9,912.22
Havekes, Robert	Research Assistant - Project 3	Penn	Year 4 - 59%	$31,640.84
Hernandez, Pepe	Post-doc - Project 3	Penn	Year 3 - 23%; Year 4 - 5%	$12,056.87
Herrington, John	Co-Investigator - Project 3	CHOP	Year 1 - 21.25%; Year 3 - 18.25%	$41,712.68
Hussein, Ayan	Summer Undergrad - Project 6	Penn	N/A – Hourly paid	$2,835.00

Iadarola, Suzannah	Post-doc - Project 5	CHOP	Year 4 - 16.5%	$6,586.30
Imielinski, Marcin	Research Assistant - Project 1	CHOP	Years 1-2 - 50%	$22,037.97
Ingalhalikar, Madhura	Post-doc - Project 5	Penn	Year 2 - 45%; Year 3 - 19%	$39,571.00
Ji, Xiao	Student - Project 2	Penn	Year 3 - 16%	$1,386.00
Johnson, Donielle	Minority Training Program Post-Bac - Project 4 & 6	CHOP	Year 3 - 100%; Year 4 - 25%	$50,769.93
Katz, Julia	Student - Project 3	Penn	Year 3 - 26%; Year 4 - 100%	$4,459.85
Kember, Rachel	Post-doc - Project 2	Penn	Year 4 - 50%	$23,053.50
Kenworthy, Charles	Research Assistant - Project 3	Penn	Year 3 - 11%	$3,442.13
Kreibich, Arati Sadalge	Postdoctoral Researcher and then Research Associate - Project 3	Penn	Year 1 - 8%; Year 3 - 29%; Year 4 - 76%	$63,206.08
Lanza, Matthew	MEG Research Assistant - Project 5	CHOP	Year 3 - 24.75%; Year 4 - 33%	$28,018.06
Le, Loan	Behavioral Research Assistant - Projects 4 & 5	CHOP	Year 4 - 25%	$10,803.99
Lemma, Maria L.	Research Technician - Project 1	CHOP	Years 1-3 - 40%	$79,140.72
Letzen, Janelle	Minority Training Program Student - Projects 4, 5 & 6	CHOP	Year 1 - 100%; Year 2 - 100%; Year 3 - 8.25%	$83,057.56
Lindquist, Ingrid	Student - Project 2	Penn	Year 1 - 10.5% Year 2 - 36.4%	$6,948.60
Liu, Rui	Post-doc - Project 2	Penn	Year 1 - 27.7% Year 2 - 22.2%	$18,637.29
Liu, Yunxian	Research Assistant - Project 3	Penn	Year 1 - 75%; Year 2 - 28%; Year 3 - 70%	$39,029.39
Mandell, David	Co-PI - Project 4	Penn	Year 2 - 6%; Year 3 - 5%; Year 4 - 2%	$20,527.43
Marcus, Steven	Statistician - Project 3, 4, & 5	Penn	Year 2 - 12.7%; Year 3 - 4.7%; Year 3 - 13.6%	$65,209.99
McDermott, Meghan	Behavioral Research Assistant - Projects 4 & 5	CHOP	Year 3 - 40%; Year 4 - 26.75%	$31,044.05
McVey, Alana	Behavioral Research Assistant - Projects 4 & 5	CHOP	Year 4 - 25%	$10,168.60
Mesaric, Julianne	Recruitment Assistant - Project 4	CHOP	Year 2 - 5%; Year 3 - 25%	$6,920.12

Mosner, Maya	Behavioral Research Assistant - Projects 4 & 5	CHOP	Year 3 - 41.75%; Year 4 - 4.25%	$18,186.15
Nair, Aarti	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 1 - 25%; Year 2 - 17.5%	$46,427.42
Pandey, Juhi	Clinician - Project 4	CHOP	Years 1-3 - 33%; Year 4 - 12%	$97,187.21
Parish-Morris, Julia	Post-doc - Eye-tracking, Project 4	CHOP	Year 1 - 7.5%; Year 2 - 36.25%	$11,442.06
Pisani, Cara	Clinician - Project 4	CHOP	Year 4 - 12.5%	$6,535.20
Prabhakar, Preeti	Database Manager - Projects 4 & 5	CHOP	Year 3 - 23.25%	$31,642.16
Puleo, Connor	Grad Fellow - Project 4	CHOP	Year 3 - 81.25%; Year 4 - 6.25%	$26,247.36
Rai, Reena	Research Assistant - Project 3	Penn	Year 1 - 35%	$7,563.83
Riley, Meghan	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 2 - 31.25%; Year 3 - 25%	$23,726.61
Roberts, Timothy	Co-PI - Project 5	CHOP	Years 1-4 - 10%	$93,300.43
Schoch, Hannah	Grad Fellow - Project 3	Penn	Year 2 - 8%; Year 3 - 6%	$4,561.68
Schultz, Robert	PI - Center, Projects 4 & 5	CHOP	Year 1-3 - 25%; Year 4 - 9.25%	$197,620.43
Shin, Christine	fMRI/DTI/sMRI Research Assistant - Project 5	CHOP	Year 1 - 25%; Year 2 - 5%	$9,059.42
Sridhar, Srijani	Student - Project 2	Penn	Year 4 - 30%	$2,564
Stephens, Nicole	Investigator - Project 4 & 6	Lincoln University	Years 1-2 - 14%	$25,880.00
Thompson, Rebecca	Post-doc - Project 4	CHOP	Year 2 - 45.75%; Year 3 - 37.5%	$39,632.59
Tian, Lifeng	Bioinformatics Specialist - Project 1	CHOP	Year 3 - 20%	$14,385.79
Tonge, Natasha	Minority Training Program Student - Projects 4, 5, & 6	CHOP	Year 3 - 100%; Year 4 - 75%	$72,976.48
Toskala, Elina	Scientist - Project 1	CHOP	Year 4 - 1%	$1,848.92
Tyree, Kareem	Minority Training Program Post-Bac - Projects 3, 4, & 6	Penn	Years 2-4 -100%	$61,818.52
Verma, Ragini	Co-Investigator - Project 5	Penn	Year 2 - 10%; Year 3 - 4%; Year 4 - 2%	$26,586
Vijayvargiya, Neha	Student - Project 3	Penn	Year 3 - 99%	$2,596.60

Wadhawan, Samir	Post-doc - Project 2	Penn	Year 1 - 40.4% Year 2 - 76.1% Year 3 - 100% Year 4 - 100%	$134,921.67
Wang, Kai	Scientist - Project 1	CHOP	Years 1-2 - 5%	$4,973.63
Wang, Xuexia	Post-doc - Project 2	Penn	Year 1 - 15.4%	$5,738
Wang, Ying	Post-doc - Project 5	Penn	Year 1 - 21%; Year 2 - 50%; Year 3 - 52%	$53,700.00
Wong, Margaret	Research Assistant - Project 3	Penn	Year 2 - 25%; Year 3 - 38%; Year 4 - 80%	$25,638.43
Worley, Julie	Post-doc - Project 4	CHOP	Year 4 - 21.25%	$10,710.27
Wu, Zhi-liang	Scientist - Project 1	CHOP	Years 2-4 - 5%	$30,064.79

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	Institution	% of Effort on Project
Chevallier, Coralie	Co-Investigator - Project 4	CHOP	Years 3-4 - 50%
deMarchena, Ashley	Intern - Project 4	CHOP	Year 4 - 20%
Georgi, Benjamin	Post-doc - Project 2	Penn	Year 1 - 5%
Guy, Lisa	Clinician - Project 4	CHOP	Year 2 - 32.5% Year 3 - 50% Year 4 - 18%
Herrington, John	Co-Investigator - Project 5	CHOP	Year 4 - 5%
Hou, Cuiping	Research Assistant - Project 1	CHOP	Years 1-4 - 2.5%
Hsin, Olivia	Post-doc - Project 4	CHOP	Year 3 - 80% Year 4 - 50%
Kim, Cecelia	Lab Manager - Project 1	CHOP	Years 1-4 - 2.5%
Khan, Munir	Research Assistant - Project 1	CHOP	Years 1-4 - 5%
Levy, Susan E.	Medical Director - Project 5	CHOP	Years 1-4 - 5%
Li, Mingyao	Collaborator - Project 2	Penn	Years 1-4 - 5%
Maxwell, Christina	Post-doc - Project 5	CHOP	Year 3 - 50% Year 4 - 50%
Miller, Judith	Clinical Training Director - Project 4	CHOP	Year 2 - 25% Year 3 - 25% Year 4 - 5%
Otieno, Frederick	Research Assistant - Project 1	CHOP	Years 1-4 - 2.5%
Parish-Morris, Julia	Post-doc - Eye-tracking, Project 4	CHOP	Years 3-4 - 20%

Prabhakar, Preeti	Database Manager - Projects 4 & 5	CHOP	Year 4 - 30%
Rump, Keiran	Post-doc - Project 4	CHOP	Year 2-3 - 50%
Ryan, Tiffany	Clinic Coordinator - Project 4 & 5	CHOP	Years 1-3 - 70% Year 4 - 30%
Thomas, Kelly A.	Research Assistant - Project 1	CHOP	Year 1-4 - 5%
Voight, Benjamin	Collaborator - Project 2	Penn	Year 4 - 5%
Winder, Breanna	Intern - Project 4	CHOP	Year 4 - 20%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Agilent 2100 Electrophoresis Bioanalyzer *(Project 2)*	This equipment provided the researchers with a means to size, quantify, and quality control DNA and RNA samples for Project 2. It has also allowed for the preparation of libraries for exome and whole genome sequencing.	$16,285.15
Avisoft Bioacoustics Software *(Project 3)*	This software allowed the researchers to begin to measure ultrasonic vocalizations, an important aspect of social communication, in the mouse models relevant to autism. Having this software helped the researchers in Project 3 leverage this grant to obtain additional funding to study mouse models of autism, including funding from the Simons Foundation (see item 11, Leveraging of Additional Funds).	$4,265.25 (total cost was $8,530.50, however, the expense was shared with a non-PA funded project)
Data analysis computer *(Project 3)*	Computer was used to assist with data analysis	$699.50

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X____ No_____

If yes, please indicate the source and amount of other funds:

Projects 1 & 4:
Lurie Autism Foundation: $400,000 per year for 5 years (Co-PIs: Hakonarson/Schultz)

Projects 2, 3, 5, & 6:

None.

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Project 1:				
Role of GABA and mGluR gene networks in autism pathogenesis (PIs: Schultz & Hakonarson)	☐NIH ☐Other federal ☑Nonfederal source (specify: Lurie Autism Foundation)	April 2011	$1M	$1M
Project 2:				
None				
Project 3:				
Neural and behavioral mechanisms of sociability deficits in Pcdh10 mutant mice (PI: Brodkin)	☑NIH: R01 ☐Other federal ☐Nonfederal source	June 2012	$1,250,000	Not funded
Developing Pcdh10 conditional transgenic mouse lines for studies of sociability (PI: Brodkin)	☑NIH: R21 ☐Other federal ☐Nonfederal source	October 2012	$270,000	Not funded
Sociability deficits and pharmacologic rescue in	☑NIH: R01 ☐Other federal	October 2012	$1,250,000	Not funded

Pcdh10 mutant mice (PI: Brodkin)	☐Nonfederal source			
Imaging glutamate-GABA balance to predict treatment response in autism models (PIs: Brodkin & Poptani)	☑NIH: R01 ☐Other federal ☐Nonfederal source	March 2013	$600,000	Pending—not reviewed or funded yet
Comprehensive phenotyping of mouse models of autism (PI: Abel)	☐NIH ☐Other federal ☑Nonfederal source (specify: Simons Foundation)	October 2011	$507,710	$507,710
Project 4:				
Characterizing IQ Impairments in ASD and testing their genetic foundations (PI: Schultz)	☐NIH ☐Other federal ☑Nonfederal source (specify: Simons Foundation & Nancy Lurie Marks Foundation)	March, 2012	$481,886	$481,886
Biobehavioral markers of Anxiety in Autism Spectrum Disorders (PIs: Herrington & Schultz)	☐NIH ☐Other federal ☑Nonfederal source (specify: Shire Pharmaceuticals)	February 2012	$2M	$2M
Toward Outcome Measurement of Anxiety in Youth with Autism Spectrum Disorders (Subcontract to CHOP; PI: Schultz)	☑NIH: R01 ☐Other federal ☐Nonfederal source	February 2012	$994,878	$902,300
Role of GABA and mGluR gene networks in autism pathogenesis (PIs: Schultz & Hakonarson)	☐NIH ☐Other federal ☑Nonfederal source (specify: Lurie Autism Foundation)	April 2011	$1M	$1M
Autism Treatment Network*	☐NIH ☐Other federal ☑Nonfederal source (specify: Autism Speaks)	July 2010	$420,000	$420,000

State and Trait Aspects of Social Motivation in Autism Spectrum Disorders (PI: Chevallier)	☑NIH: R21 ☐Other federal ☐Nonfederal	October 2012	$275,000	Not funded
Social Motivation and Reward Mechanisms in Autism (PI: Schultz)	☑NIH: R01 ☐Other federal ☐Nonfederal	June 2012	$2,007,829	Not funded
Social Motivation in ASD: Mechanisms, Treatment, and Community Translation (PI: Schultz)	☑NIH: P50 ☐Other federal ☐Nonfederal	December 2011	$11,712,824	Not funded
Project 5:				
Novel computational methods for higher order diffusion MRI in autism (PI: Verma)	☑NIH: R01 ☐Other federal ☐Nonfederal	February 2010	$3,561,199	$3,561,199
Quantifiable markers of ASD via multivariate MEGDTI combination (PI: Verma)	☑NIH: R01 ☐Other federal ☐Nonfederal	July 2012	$275,000	$275,000
Project 6:				
None				

*This grant benefited from the large clinical infrastructure created for this Health Research grant. It is a large undertaking to recruit and study 678 children and adolescents and once established, this made our Center quite unique (one of a very small handful in the world capable of conducting single site large autism studies). We've leveraged this infrastructure for new grants, showing a track record for large sample recruitment and an established Clinical team.

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X____ No_____

If yes, please describe your plans:

Project 1:
The researchers for Project 1 are planning to submit two grants to the NIH: 1) to characterize the role of MSN antisense function (*MSNP1AS*; moesin pseudogene 1, antisense) at the 5p14 locus in interfering with regulation and action of the moesin protein on the X chromosome, in cell based assays and animal models, and 2) to study autism clinical development through

GABA and glutamate/mGluR mechanisms.

Project 2:
The researchers of Project 2 plan to submit a proposal in October 2013 in response to the NIH Program Announcement PAR-13-231. The grant will be entitled, "The role of essential genes in ASD," and will include experimental validation of rare exonic and regulatory (non-coding) variants identified in the CURE grant Project 2.

Project 3:
The researchers of Project 3 intend to apply for additional NIH and private foundation grants to study amygdala development and function in protocadherin 10 +/-mice, and the role of amgydala functioning in social behavior development relevant to autism, as well as grants to identify pharmacologic agents that can rescue sociability and amygdala function in this mouse line. The researchers also intend to apply for NIH and private foundation grants to continue study of the Cdh10 conditional knockout line.

Project 4 & 5:
The focus of the CURE research - studying the relationships genetic, neurobiological, imaging, behavioral and clinical features of ASD - will remain the scientific focus of the researchers' work. In fact, the newest strategic plan of the National Institute of Mental Health (NIMH) explicitly notes that studying the relationships between genes, brain and phenotypic processes is necessary to in order to clarify the underlying causes of ASD. And the core mission of the Center for Autism Research is to characterize the causes of ASD so that more effective treatments can be devised. Most of the researchers funded through this CURE Grant are either fully dedicated or largely dedicated to autism research, and depend on grant funding through the NIMH. To effectively compete for grant funds for research through the NIMH and other granting agencies with similar priorities, the researchers will continue this line of work. Project 4 led to submission of an R01 in February of 2012, which was scored and will be resubmitted once more papers from Project 4 are in press. The researchers plan to also submit grants on genetics and brain imaging based on expected results from final analyses in Project 5. The work begun with the CURE Center grant will provide the data, publication track record, and scientific motivation (e.g., new hypotheses) needed for many future ASD grant applications.

Project 6:
While the researchers have no immediate plans to apply for new grant funding to continue the minority training program, the successful training model that the researchers developed as part of the CURE Center Grant will be implemented as a continued component of CAR's general (minority and non-minority) undergraduate, post-baccalaureate, and graduate training on autism spectrum disorder. Moreover, the researchers who participated in this CURE Center grant also participate in a number of NIH funded graduate and post-doctoral training programs via NIH's "T32" mechanism. Experience with this minority-training program has been valuable and can provide a model to enhance elements of minority training in these other programs, which also emphasize the need for aggressive strategies in order to expand the pool of minority participants in research. This is a critical mission in light of the poor representation of certain minorities, and it requires that research organizations be as proactive

as possible. The consortium of undergraduate and graduate student research training programs that includes LEND, McNair, PennPREP, Temple University, and Lincoln University can continue to serve in this goal.

12. Future of Research Project. What are the future plans for this research project?

Project 1:
The researchers plan to: 1) characterize the role of MSN antisense function (*MSNP1AS*; moesin pseudogene 1, antisense) at the 5p14 locus in interfering with regulation and action of the moesin protein on the X chr, in cell based assays and animal models. This project presents a series of experiments designed to study the mechanism by which *MSNP1AS* blocks the encoding of moesin and is tailored towards resolving the interference, thereby potentially enabling new/novel therapy for autism in patients who carry the 5p14 locus mutation. 2) Characterize GABA and glutamate autism gene networks in preparation for clinical trials. The researchers existing genetics data strongly points to a role for both cell adhesion gene networks and GABA/glutamate gene networks. The former is addressed in #1, whereas the latter have direct treatment implications as there is a drug candidate (NS-105) that appears to be able to restore the glutamine function in persons with ASD and disruption of the glutamine-signaling pathway. In addition, there are drug candidates such as Topamax that may restore the GABA pathway function in persons with ASD who have GABA-network disruption.

Project 2:
The researchers' immediate plan is finalize analyses integrating genetic findings with the phenotypic data for publications (collaboration between Drs. Bucan, Hakonarson and Schultz).

Project 3:
The researchers plan to continue phenotype characterization of the *Cdh10* conditional knockout mouse line, and to elucidate the mechanisms of reduced sociability in the *Pcdh10*+/- mouse line, and mechanisms of sociability rescue in this line. Based on the data generated in this project, we hypothesize that sociability deficits of *Pcdh10*+/- mice are attributable to deficits in glutamatergic signaling in neural circuits involving the basolateral amygdala. The researchers plan to carry out future studies to test this hypothesis, and to test the efficacy of pharmacologic agents that modulate glutamatergic signaling in rescuing social behaviors in this mouse line.

The researchers also plan to extend this project to translational studies in humans with ASD, testing the association of protocadherin and cadherin gene variants in human ASD, and testing the efficacy of pharmacologic agents that modulate glutamatergic signaling, such as d-cycloserine, in treatment of social behavior deficits in ASD.

Project 4 & 5:
As described in context and with greater detail in responses to Question 17, the researchers plan to continue data analyses and publication of the large amount data collected in Projects

4 and 5. Effort over first 4 years of this work focused more on data collection than analyses; effort will now be fully dedicated toward finishing analyses and publications. The phenotypic and brain imaging databases established by Projects 4 and 5 are very valuable scientific resources that will be mined for several years. Project 4 will focus in genotype-phenotype analyses. Similarly, Project 5 will integrate genetic data into unimodal analyses. Project 5 will also focus on multimodal pattern analyses for publication. In addition to generating new peer review publications, the data from Projects 4 and 5 will be used for preliminary data in support of new grant applications over the next few years.

Project 6:
This training program project does not have scientific goals. However, as described in response to question 11(B), the minority-training infrastructure will continue at the researchers' home institutions for other training programs.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	7	1	1	6
Female	24	2	12	13
Unknown				
Total	**31**	**3**	**13**	**19**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	2			2
Non-Hispanic	29	3	13	17
Unknown				
Total	**31**	**3**	**13**	**19**

	Undergraduate	Masters	Pre-doc	Post-doc
White	16	1	11	11
Black	4	1		
Asian	9	1	2	6
Other	1			2
Unknown	1			
Total	**31**	**3**	**13**	**19**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes___X___ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

Jennifer Bush, AB, Princeton University (Project 4)
Coralie Chevalier, PhD, Institute of Psychiatry, King's College, London, England (Project 4)
Terisa Gabrielsen, PhD, University of Utah (Project 4)
Benjamin Georgi, PhD, Freie Universität, Berlin, Germany (Project 2)
Ivy Giserman, BA, University of Rochester (Project 4)
Lisa Guy, PhD, Emory University (Project 4)
Olivia Hsin, PhD, Harvard Medical School, Massachusetts General Hospital (Project 4)
Hee-Won Kang, BA, Columbia University (Project 4)
Rachel Kember, PhD, Imperial College, Medical Research Council, London, England (Project 2)
Judith Miller, PhD, University of Utah (Project 4)
Keiran Rump, PhD, University of Miami (Project 4)
Julie Worley, PhD, Kennedy Krieger Institute (Project 4)
Michelle Villalobos, PhD, Yale University (Project 4)

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Project 1:
The research support led to numerous new collaborations both institutional and domestic/ international and enhanced the reputation of the research program.

Project 2:
The research support led to 3-4 publications (1 published, 2-3 in preparation); a talk by Wadhawan at the 2012 IMFAR in Toronto and poster presentation at the "Cell Symposia: Autism," 2011 SFN satellite meeting on Autism Spectrum Disorders and a talk by Bucan at the 2011 SFN meeting, Washington D.C.; an NIH grant in preparation on the "Mutational load in essential genes in Autism Spectrum Disorders" (submission planned for October 2013); and the development of infrastructure for the analysis *of whole genome sequence in ASD.*

Project 3:
By identifying a mouse model (*Pcdh10*+/- mice) with both etiological and face validity for ASD, as well as a pharmacologic agent (d-cycloserine) that can rescue sociability deficits in this model, the researchers have developed an important new resource for basic research in animal models, as well as translational research on ASD that ultimately promises to improve care for the largely treatment-refractory social impairments of ASD.

Projects 4 & 5:
The CURE grant was the major source of research funding for the Center for Autism Research (CAR) and its collaborating partners on this grant over the last 4 years. Funding came just as this new Center for Autism Research (CAR) was being established at CHOP and Penn (CAR was officially launched in the spring of 2008 after Robert Schultz was recruited to CHOP/Penn to serve as its first Director). CURE funding helped galvanize the start up process, allowing CAR to build out its clinical and research capacities with new personnel, and establish operating procedures for clinical research. Writing and executing the CURE grant was also very helpful to CAR and the broader autism research community at CHOP and Penn as it brought together the team of PIs and co-investigators, solidifying new relationships that can be relied on for future research collaborations. CURE funding, thus, has been extremely helpful for bringing together the autism research community, including new faculty recruits (e.g., Drs. Herrington, Yerys, and Miller), at CHOP and Penn.

Project 6:
The minority training program has fostered and enhanced diversity in the clinical research settings of CHOP and Penn, and served to assist underrepresented minorities acquire the training needed to successfully be admitted to graduate school, and to find professional employment in the autism field.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X____ No_____

If yes, please describe the collaborations:

Project 1:
Dr. Hakonarson and colleagues have established research collaborations in autism as a result of this grant at The Children's Hospital in Melbourne Australia, and Queens University of Belfast in N-Ireland as well as The University of Connecticut Health Center in Farmington CT. The researchers have also enhanced collaborative efforts with the AGP consortium as a result of this research.

Project 2:
Dr. Bucan has established collaboration with Dr. Toru Takumi (Riken Institute for Brain Research, Japan) and with Dr. Matthew Dalva (Jefferson University, Philadelphia) on the experimental validation of variants found in Project 2.

Project 3:
This research has led to a new collaboration with Dr. Joshua Corbin, Associate Professor of Pediatrics, Pharmacology, and Physiology at the George Washington University

School of Medicine and Health Sciences, who will work with the Brodkin lab in assessing amygdala development in *Pcdh10*+/- mice.

Project 4 & 5:
The researchers started collaborating with researchers in the new Geisinger-Bucknell Autism and Developmental Medicine Center in Lewisburg, PA. This new collaboration is based on knowledge gained from Projects 4 and 5. Drs. David Ledbetter, David Evans and colleagues are beginning new imaging and phenotypic studies at the Geisinger-Bucknell Autism Center, with assistance from researchers at CAR.

This research has also led to new collaborations on phenotypic-genetic relationships with Dr. Bernie Devlin at the University of Pittsburgh, which is funded by a grant from the Simons Foundation.

Project 6:
None.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes___X_____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

Project 1:
Dr. Hakonarson and his lab have given lectures on the project and its results to the community in Philadelphia, which has inspired patients and their families to participate in research at CHOP.

Project 2:
Drs. Bucan and Kember participated in the Philadelphia Science Festival, describing their work on genetic studies of Autism Spectrum Disorders.

Project 3:
None.

Project 4 & 5:

During the course of CURE grant funding, the outreach/recruitment team at the Center for Autism Research attended over 350 community events sponsored by educational and autism focused organizations. At these events CAR personnel (a) provided educational materials on autism; and (b) described current research, clinical and outreach activities at CAR so that families affected by ASD and health professionals and educational specialists working with ASD might become more involved. CAR clinicians, outreach staff, and scientists were also frequently sought out to present to parent groups about early warning signs of autism, treatments, and ways to promote tolerance, inclusion, and acceptance of children with ASD. Presentations have been given at camps, schools, YMCA events, mom's clubs, parenting organizations, and other community organizations.

To help increase study recruitment, CAR partnered with the Philadelphia Eagles football organization. Each of the last four years, CAR and the Eagles have co-sponsored an autism awareness event – *Huddle Up for Autism* – for the community. CURE grant participant recruitment directly benefited, as there was a research table at the event taking family contact information and describing study details and soliciting participation. *Huddle Up for Autism* has become extremely popular in the autism community, with attendance of 5,000 people (the cap for this event) each of the last three years. Attendance is free and attendees are able to tour the Eagles' stadium, including the locker rooms, participate in events on the field (e.g., field goal kicking), and meet players, the mascot and cheerleaders. There are also a variety of carnival type activities and games.

Each year, CAR holds about 10 public lectures (as part of CAR's Distinguished Lecture Series or DLS) on autism to help make research more accessible to the community (see answer for Project 6 to question 17 for a listing of the lectures). Over 50 lectures have been held during the course of CURE grant funding, on topics ranging from immunology to genetics to behavioral interventions. Many of the DLS speakers present two presentations – one for the scientific community and one for the general public. Presentations geared for the community are held in the evening to provide an opportunity for families and community autism service providers to attend. These presentations are two hours long and are usually filled to capacity (approximately 100+ guests). Parents and area teachers and clinicians who attend are engaged and enthusiastic learners at CAR's lecture series, and many of the attendees go on to participate in research at CAR and/or to tell others about CAR's work. The lecture series has been successful in attracting a variety of community service providers, who can take back what they learn at DLS and thus help to disseminate the latest research and autism best practices into community settings. Continuing education credits are available to doctors, nurses, social workers, psychologists, and teachers who attend. The lecture series is funded by CHOP as part of CAR's mission, but also explicitly to form and maintain a partnership with families affected by autism and with providers of ASD services in order help with research recruitment. As the largest study at CAR (by far) over the last 4 years, the CURE grant has been a driving force for this lecture series.

In addition, CAR personnel started their Next Steps workshops in part in order to facilitate study recruitment, but also because outreach and education is a core component of CAR's mission. As described more fully under Project 6 for question 17, the Next Step's program occurs 6 to 8 times per year and is attended by parents of youth with ASD as well as professionals needing more education about ASD. It is a day long, multidisciplinary team based workshop presentation, with a parent panel at the end of the program. Attendance is capped at 50 per event, and it always books out. It has been run for the past 3 years and will continue on.

Because many families – particularly those living with autism – may not be able to attend community events, CAR makes use of online opportunities. CAR's website is now being redesigned to improve access to autism information and resources. An online autism "Roadmap" has recently been funded by a private donation, which will include a Pennsylvania resource directory and information on obtaining and understanding an ASD diagnosis, navigating the complex service systems for ASD (including the behavioral health and education systems), and managing co-morbid conditions (for example, ADHD, anxiety, and sleep disorders). CAR also has a regular eNewsletter, which is sent to almost 10,000 families and professionals, and includes research highlights and events and articles of interest.

Project 6:
There has not been community involvement per se, but conducting Project 6 did enhance the researchers' involvement with collaborating Universities (Temple and Lincoln) and with other minority training programs at Penn.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a

performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

PROJECT 1: CHARACTERIZING THE COMMON INTERGENIC VARIANT AT THE 5p14 locus IN ASD. (PI: HAKON HAKONARSON, MD, PhD)

In 2009, Dr. Hakonarson and colleagues published a landmark finding, where the researchers identified and replicated the first common genetic variant in autism, an intergenic region on 5p14, using GWAS (Wang et al, 2009). Project I of this research grant was subsequently designed to identify and characterize the causal variant(s) in *the CDH9/CDH10* intergenic region on 5p14 to further address the roles of the variants in ASD pathogenesis. The following Specific Aims and associated hypotheses from the original application were subsequently pursued:

Specific Aim 1.1: To isolate the chromosomal region corresponding to the linkage disequilibrium block harboring an ASD risk locus identified through GWA plus the two flanking cadherin genes in 120 individuals (60 cases and 60 controls) using a sequence enrichment protocol based on a novel Region Specific Extraction method. The researchers will isolate the genomic region corresponding to the linkage disequilibrium block of the genome where the GWA SNP signals reside and the two flanking cadherin genes in 60 ASD cases and 60 matched controls. These templates will be used in Aim 2 for ultra-high-throughput parallel sequencing.
Hypothesis 1.1: The researchers hypothesize that re-sequencing of the *CDH9/CDH10* intergenic region on 5p14 will identify novel variants that contribute to the pathogenesis of ASDs.

Specific Aim 1.2: To apply massively parallel sequencing to the regions obtained as a consequence of the isolation procedure and assemble the resulting sequence data with bioinformatic tools in order to catalogue all variation in the isolated DNA sequences that contribute to the genetic risk of ASD. The targeted regions will be subjected to highly parallel sequencing using the Illumina Genome Analyzer (IGA) system to catalogue the existing variation in the region in a cost-effective and high throughput manner. The researchers will capture both common variants in linkage disequilibrium with the associated SNPs and rare variants that may predispose to ASD independently. The researchers will use vendor-specified software to assemble the sequence data generated on the IGA system. These sequences then will be aligned to the latest public assembly of the human genome using open source third party

applications. All variation in the isolated DNA sequences will then be systematically catalogued. *Hypothesis 1.2*: The researchers hypothesize the variants identified by re-sequencing of the DNA fragments generated for the *CDH9/CDH10* intergenic region on 5p14, using Regions Specific Capturing, will Include the disease-causing variants (i.e., smoking gun mutation) at this locus that underlie ASDs.

Specific Aim 1.3: To genetically validate the short-listed variants identified in Aims 1 and 2 by genotyping them in 3,000 ASD cases and 3,000 control subjects without ASD of whom approximately 90% are of Northern European and 10% of African ancestry. A cohort of ~3000 Caucasian ASD cases (2600 existing and 400 new recruits) and 3000 controls will be utilized in order to genetically validate, through targeted genotyping using the Illumina GoldenGate platform, the short-listed variants obtained from the discovery Caucasian subjects that were re-sequenced. Approximately 90% of the ASD cases and controls are of Northern European ancestry and 10% of African ancestry.
Hypothesis 1.3: The researchers hypothesize that they will validate the short-listed variants enriched in the discovery cases in the expanded Caucasian ASD cohort and that the most significant risk variants in the Caucasian cohort will also show association with ASD in the newly recruited African American cohort

METHODS:
The researchers used two complementary methods to identify all non-coding variants within the 5p14.1 region as well as the coding variants in the flanking cadherin genes, CHD9 and CHD10, residing at the locus. In addition, all coding SNPs in 97 cadherin/protocadherin genes that were enriched for SNP signals in the GWAS study by Wang et al (2009) in the context of gene network analysis were also included. In doing this, the researchers first develop a custom Agilent SureSelect panel, by capturing all annotated exons for these 99 candidate genes based on RefSeq gene annotations. These genes include those identified from published candidate gene association studies, as well as known neuronal cell adhesion genes (such as cadherins, neurexins, neuroligins, NCAMs) all of which were enriched in our GWAS gene-based network analysis (Wang et al, 2009). The researchers used this panel to sequence 144 patients with autism by the Illumina Genome Analyzer and identified over 100 rare coding mutations in these genes. The researchers used ANNOVAR (Wang et al, 2010) to annotate the functional consequences of these mutations, and prioritized a list of functionally important (predicted deleterious) non-synonymous coding variants for follow up genotyping.

The researchers next designed a custom RainDance panel to amplify the LD block within the 5p14.1 region, using multiplex droplet PCR reactions. Specific criteria for the PCR primer selection include: Tm between 57 and 59, product size between 200-600bp, at least 50bp overlap between adjacent amplicons. Dr. Hakonarson and colleagues sequenced the PCR products by SOLID platform, and prioritized a list of rare non-coding variants for follow up.

From the variants identified using the above methods, the researchers designed a custom Veracode genotyping array. The array holds a maximum of 384 SNP markers, and 25% ancestry informative markers were included in the design, given that rare variants association are known to be affected by population stratification. Illumina later converted the Veracode array to a GoldenGate custom-based genotyping array as there were difficulties with performance

measures on the Veracode array resulting in SNP failures when genotyped. All such failures were re-done on the Goldengate array. The researchers genotyped our existing ASD cohort using these two array platforms to identify coding and non-coding variants that may be associated with ASDs. A haplotype-based approach was used in the design to ensure all independent novel variants were captured for genotyping. This included all rare variants (less than 1% MAFs) and one or more representative common variants for each haplotype.

The breakdown of samples was as follows for the two gene chip platforms:

Goldengate		Veracode	
384	markers	384	markers
4350	individuals	1742	individuals
2134	cases	966	cases
2216	controls	776	controls

A total of 3199 cases and 2992 controls were genotyped in total. Initial Quality Control (QC) of samples and data resulted in 148 cases and 123 controls being removed for poor genotyping quality (on either platform). This left us with 2954 cases and 2869 controls with good genotyping quality (0.994) for the final analysis, broken down as follows.

Goldengate		Veracode	
4233	individuals	1590	individuals
2073	cases	881	cases
2160	controls	709	controls
0.99426	genotyping rate	0.993835	genotyping rate

In order to control for genomic inflation, Dr. Hakonarson and colleagues analyzed each ethnicity separately as case/control, using the AIMS markers they included in the chip design to derive ethnicity which resulted in the following breakdown: AWS 7; CEU 2917; CHB 11; CHD 13; GIH 213; JPT 12; LWK 32; MEX 219; MKK 148; NA 455; TSI 2050; and YRI 15. Since the majority of the samples are CEU (European ancestry in Utah) or TSI (European in Italy), the analysis was driven using these subsets.

Follow-up Sequencing.
For more extended validation and analysis of the targeted sequencing results with follow-up genotyping, the researchers generated whole exome sequencing data on nine autism families (proband/parents) and whole genome sequencing data on one family. Those samples were similarly analyzed using our high-throughput sequencing pipeline at CAG which is designed to identify and prioritize mutations the researchers uncover for follow-up and relate them to existing clinical data. The sequencing and data analysis pipeline consists of the following major steps:

1. Quality Control (QC)
The QC process is designed to detect aberrant runs, to identify potential contaminations and sample swaps, improve reads qualities, and enhance the variant call accuracy as a result. The researchers use both vendor supplied and third party tools to ensure adequate QC of the data. In addition to managing, executing, and tracking the sequencing runs, the Illumina Sequence

Control Software (SCS) with Real Time Analysis (RTA) allows the real time monitoring of the sequencing quality and detecting problems. Of the many third party tools available, FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) provide a useful collection of QC metrics.

2. Short reads alignment and pre-processing

The key steps of this process include:
a) Pre-processing of raw reads outputted from sequencing instruments;
b) Mapping of the reads against the Human Genome Project reference using BWA (Li and Durbin, 2009);
c) Remove duplicates using Picard (http://sourceforge.net/projects/picard)
d) Local realignment and variant refinement: re-calibration and re-alignment of reads at indels using GATK (DePristo et al., 2011).

3. SNV and Indel call

There are quite a few tools for variant calls. The researchers consider the following tools for calling single nucleotide variants (SNVs):

a) GATK http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit
b) SAMtools http://samtools.sourceforge.net/samtools.shtml
c) SNVer http://snver.sourceforge.net/ (Developed by CAG)

Each tool has its own pros and cons. The researchers observe sizable variance between different tools. Dependent on the tradeoff between power and accuracy, the researchers take the union (maximizing power) or the intersection (maximizing accuracy) of the variant call sets generated by each program. After variant calls are made, Ti/Tv ratios are used to evaluate the overall quality of the variant call set. Ti/Tv ratios for known and novel variants are reported separately.

The reserachers use GATK and SAMtools for small indel call as SNVer doesn't have full support for indel at the time being. Caution is taken for indel call since it is more challenging and few tools can generate reliable calls based on our experience.

4. Variant annotation and prioritization

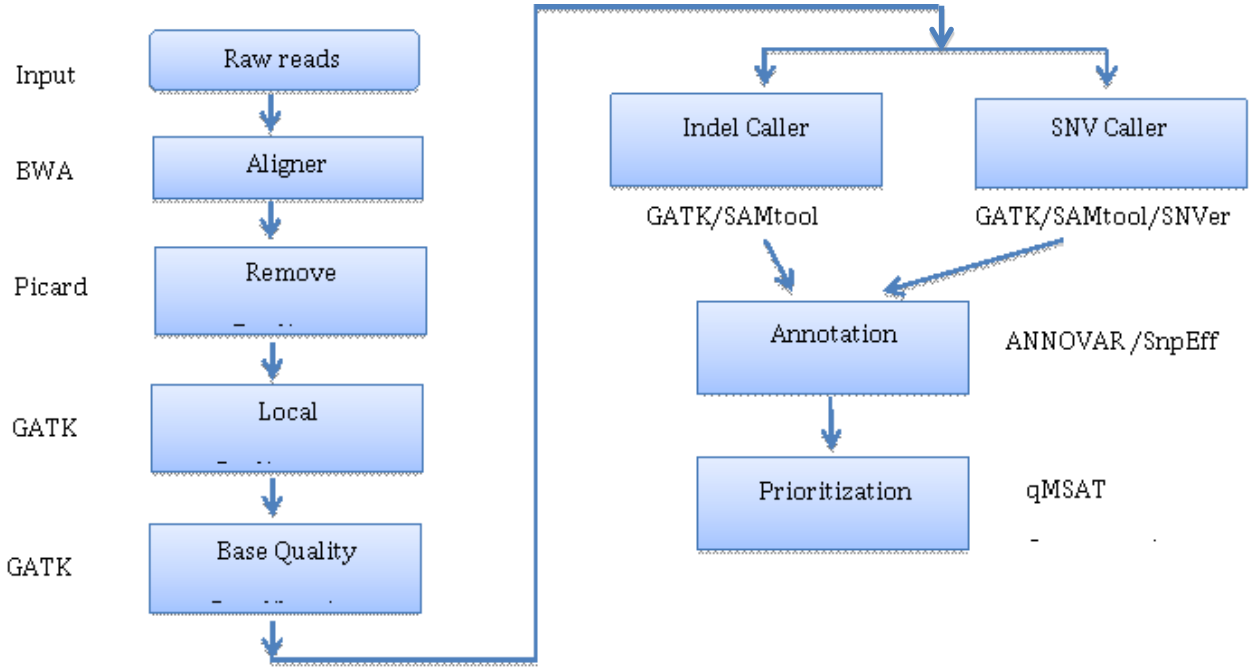
For small sample sizes, the researchers implemented SNP prioritization and pathogenic variant identification using an improved annotation-filtering method based on ANNOVAR which was developed by our laboratory (Wang et al., 2010). The researchers also use snpEff and an entirely new probabilistic disease gene finder called VAAST (Rope et al., 2011). For the identification of disease-causing mutations, the researchers initially developed an annotation-filtering procedure ANNOVAR, which functionally annotates SNPs and CNVs generated from high-throughput sequencing experiments within exonic regions. Furthermore, ANNOVAR can automatically identify variants that were previously reported in pubic databases, including the 1000 genomes project, dbSNP, NHLBI GO Exome Sequencing Project (ESP), and the Database for Genomic Variants. SnpEff is also used to validate ANNOVAR annotations.

General strategy for variant prioritizations:

1. Nonsynonymous change with damaging effect as determined by, e.g. SIFT and PolyPhen2 score
2. Conservativeness for non-coding variants, particularly in 3'UTR and 5'UTR.
3. Rareness: minor allele frequencies in lower than 0.5% in 1000 genomes and ESP exomes.

When sample size is large, the researchers use association Test to prioritize candidate variants. The researchers conduct several different association tests on the data, including (1) single variant association test (Fisher exact test or Chi-Square test) and (2) a multivariate (e.g. gene based) association test, qMSAT: http://qmsat.sourceforge.net/

If no controls are given or of a small size (n), the researchers first filter out SNPs in dbSNP132 and in the controls. After this step, published data in dbSNP (including data from 1000 Genomes project) are evaluated for the presence of the variants. The rate for each SNP variant in *m* controls from dbSNP and *n* internal control samples (*m* + *n*) are then compared to the cases using Fisher's exact test or Chi square. Variants are then ranked by their p values. A schematic overview of the workflow is provided in the figure below.



RESULTS:

Significant GWAS signals at the 5p14 intergenic locus (led by rs4307059, *P*=4.9x10; OR=1.19), suggested a functional mutation at the chr 5 locus in ASD. Thus, the research team's efforts were intended to (1) characterize this locus further by re-sequencing the region to capture the functional variant(s) involved and (2) genotype candidate SNPs in 3000 ASD cases and 3000 control samples. However, no custom-based SNP genotype using the Veracode/Goldengate panels at the 5p14 intergenic locus reached significance over and above the GWAS SNPs, suggesting that DNA variation may not be the underlying cause for the GWAS signal. It was therefore intriguing when a group of investigators at USC, let by Daniel B. Campbell, recently

reported results from a study entitled "A Noncoding RNA Antisense to Moesin at 5p14.1 in Autism". The authors used a tiling array approach and uncovered a 3.9-kb noncoding RNA that is transcribed from the region of the chromosome 5p14.1 ASD GWAS association SNPs the reseachers reported (Wang et al, 2009). The noncoding RNA was encoded by the opposite (antisense) strand of moesin pseudogene 1 (*MSNP1*), referred to as *MSNP1AS* (moesin pseudogene 1, antisense). Chromosome 5p14.1 *MSNP1AS* was 94% identical and antisense to the X chromosome transcript of *MSN*, which encodes a protein (moesin) that regulates neuronal architecture. Individuals who carry the ASD-associated rs4307059 T allele (lead SNP from our GWAS study) showed increased expression of *MSNP1AS*. The*MSNP1AS* noncoding RNA bound to *MSN*, was highly overexpressed (12.7-fold) in postmortem cerebral cortex of individuals with ASD, and could regulate levels of moesin protein in human cell lines (Kerin et al, 2012).

Taken together, these data reveal a biologically functional antisense mechanism from this locus impacting transcription of the *MSN* gene on the X-chromosome, thereby providing a biological explanation for the statistical signal at the 5p14 locus originally reported by us (Wang et al, 2009). While more work is needed to fully characterize the mechanism by which this ncRNA contributes to ASD risk, it presents a compelling mechanism in explanation of the statistical signal and why the researchers are not identifying unique disease-causing mutations or variants of greater significance at the locus through our efforts.

There were no significant SNPs either in the *CDH9* or *CDH10* exon sequences generated and genotyped. However, among the other 98 cadherin/procadherin loci the research team sequenced exons from and subsequently genotyped, one locus included 3 nsSNPs residing in the exon sequences of the *CDH26* gene that were significantly associated with ASD. While *CDH26* was enriched for association in our GWAS analysis based on gene pathway/network analysis (Wang et al, 2009) these three nsSNPs are associated with ASD for the first time. The association results are shown in following table.

CHR	BP	SNP	syn/nonsyn	MAF cases	MAF controls	P	OR	CI	CHISQ	SE	L95	U95
20	57995966	C>G	non-synonymous	0.02191	0.01243	0.00063	1.779	0.95	11.68	0.1708	1.273	2.486
20	57997541	A>G	non-synonymous	0.02161	0.01245	0.00092	1.752	0.95	10.98	0.1713	1.252	2.45
20	58009827	A>G	non-synonymous	0.03246	0.02031	0.00043	1.618	0.95	12.4	0.1379	1.235	2.12

In addition, the researchers identified three SNPs that we believe to be nsSNPs with potential impact on the function encoded by the gene. These were identified by using hg18 reference to examine the following significantly associated markers: 57995966-CDH26-exonic (C>G); 57997541-CDH26-exonic (A>G); and 58009827-CDH26-exonic (A>G). Data for each is provided below.

57995966-CDH26-exonic:

dbSNP 130: rs11086690
Reference: C
Alternate: G (non-synonymous)
1KG MAF: 0.007

Function: Missense (**C**GA) -> (**G**GA)
(r) Arg -> (g) Gly

>NP_817089.1
1 mamrsgrhps llllvlllw llqvsiidsv qqetddltkq tkekiyqplr rskrrwvitt
61 leleeedpgp fpkligelfn nmsynmslmy lisgpgvdey peiglfsled hengriyvhr
121 pvdremtpsf tvyfdvvers tgkivdtsli fnirisdvnd hapqfpekef nitvqenqsa
181 gqpifqmlav dldeentpns qvlyflisqt pllkesgfrv drlsgeirls gcldyetapq
241 ftllirardc gepslssttt vhvdvqegnn hrpaftqeny kvqipegras qgvlrllvqd
301 **r**dspftsawr akfnilhgne eghfdistdp etnegilnvi kpldyetrpa qsliivvene
361 erlvfcergk lqpprkaaas atvsvqvtda ndppafhpqs fivnkeegar pgtllgtfna
421 mdpdsqirye lvhdpanwvs vdknsgvvit vepidresph vnnsfyviii havddgfppq
481 tatgtlmlfl sdindnvptl rprsrymevc esavheplhi eaedpdlepf sdpftfeldn
541 twgnaedtwk lgrnwgqsve lltlrslprg nylvplfigd kqglsqkqtv hvricpcasg
601 ltcveladae vglhvgalfp vcaafvalav allfllrcyf vlepkrhgcs vsndeghqtl
661 vmynaeskgt saqtwsdveg qrpallicta aagptqgvkd leevppsaas qsaqarcalg
721 swgygkpfep rsvknihstp aypdatmhrq llapvegrma etlnqklhva nvleddpgyl
781 phvyseegec ggapslssla sleqelqpdl ldslgskatp feeiysesgv ps

Protein Domain: at the end of a Ca2+ binding site, mediates cell-cell contacts, thought to be essential for trans-association.

57997541-CDH26-exonic

dbSNP 130: rs28409250
Reference: A
Alternate: G
1KG MAF: 0.007
Function: Missense (A**A**T) -> (A**G**T)
(n) Asn -> (s) Ser

> NP_817089.1
1 mamrsgrhps llllvlllw llqvsiidsv qqetddltkq tkekiyqplr rskrrwvitt
61 leleeedpgp fpkligelfn nmsynmslmy lisgpgvdey peiglfsled hengriyvhr
121 pvdremtpsf tvyfdvvers tgkivdtsli fnirisdvnd hapqfpekef nitvqenqsa
181 gqpifqmlav dldeentpns qvlyflisqt pllkesgfrv drlsgeirls gcldyetapq
241 ftllirardc gepslssttt vhvdvqegnn hrpaftqeny kvqipegras qgvlrllvqd
301 rdspftsawr akfnilhgne eghfdistdp etnegilnvi kpldyetrpa qsliivvene
361 erlvfcergk lqpprkaaas atvsvqvtda ndppafhpqs fiv**n**keegar pgtllgtfna
421 mdpdsqirye lvhdpanwvs vdknsgvvit vepidresph vnnsfyviii havddgfppq
481 tatgtlmlfl sdindnvptl rprsrymevc esavheplhi eaedpdlepf sdpftfeldn
541 twgnaedtwk lgrnwgqsve lltlrslprg nylvplfigd kqglsqkqtv hvricpcasg
601 ltcveladae vglhvgalfp vcaafvalav allfllrcyf vlepkrhgcs vsndeghqtl
661 vmynaeskgt saqtwsdveg qrpallicta aagptqgvkd leevppsaas qsaqarcalg
721 swgygkpfep rsvknihstp aypdatmhrq llapvegrma etlnqklhva nvleddpgyl
781 phvyseegec ggapslssla sleqelqpdl ldslgskatp feeiysesgv ps

Protein Domain: Close to Ca2+ binding site
58009827-CDH26-exonic

dbSNP 130: rs41310817
Reference: A
Alternate: G
1KG MAF: 0.009
Function: Missense (C**A**G) -> (C**G**G)
(q) Gln -> (r) Arg

> NP_068582.2 (isoform a)

1 mkpliwtwsd vegqrpalli ctaaagptqg vkdleevpps aas**g**saqarc algswgygkp
61 feprsvknih stpaypdatm hrqllapveg rmaetlnqkl hvanvleddp gylphvysee
121 gecggapsls slasleqelq pdlldslgsk atpfeeiyse sgvps

Not part of a protein domain

> NP_817089.1 (isoform b)

1 mamrsgrhps llllvlllw llqvsiidsv qqetddltkq tkekiyqplr rskrrwvitt
61 leleeedpgp fpkligelfn nmsynmslmy lisgpgvdey peiglfsled hengriyvhr
121 pvdremtpsf tvyfdvvers tgkivdtsli fnirisdvnd hapqfpekef nitvqenqsa
181 gqpifqmlav dldeentpns qvlyflisqt pllkesgfrv drlsgeirls gcldyetapq
241 ftllirardc gepslssttt vhvdvqegnn hrpaftqeny kvqipegras qgvlrllvqd
301 rdspftsawr akfnilhgne eghfdistdp etnegilnvi kpldyetrpa qsliivvene
361 erlvfcergk lqpprkaaas atvsvqvtda ndppafhpqs fivnkeegar pgtllgtfna
421 mdpdsqirye lvhdpanwvs vdknsgvvit vepidresph vnnsfyviii havddgfppq
481 tatgtlmlfl sdindnvptl rprsrymevc esavheplhi eaedpdlepf sdpftfeldn
541 twgnaedtwk lgrnwgqsve lltlrslprg nylvplfigd kqglsqkqtv hvricpcasg
601 ltcveladae vglhvgalfp vcaafvalav allfllrcyf vlepkrhgcs vsndeghqtl
661 vmynaeskgt saqtwsdveg qrpallicta aagptqgvkd leevppsaas **g**saqarcalg
721 swgygkpfep rsvknihstp aypdatmhrq llapvegrma etlnqklhva nvleddpgyl
781 phvyseegec ggapslssla sleqelqpdl ldslgskatp feeiysesgv ps

Not part of a protein domain

Functional studies to address the impact of these variants on RNA expression and gene function in cell-based assays is underway.

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PROJECT 2: UNDERSTANDING THE CONTRIBUTION OF RARE VARIANTS. (PI: MAJA BUCAN, PhD)

Project 2 focused on the analysis of rare variants in ASD subjects recruited by CAR. This project combined experimental and computational approaches to search for a full spectrum of causal variants in ASD candidate genes. Originally, the researchers proposed to focus on a panel of 100 ASD candidate regions that were selected based on pathway analysis of high-density genotype data. With the advancement of new technologies in next generation sequencing, instead of focusing on preselected candidate regions, it became more cost effective to address the role of rare variants by whole genome sequencing of a subset of CAR recruited (and deep-phenotyped) subjects, followed by (whole) Exome SNP genotyping of all available CAR subjects. Moreover, the availability of whole genome sequences for a subset of subjects allowed for the analysis of both exonic and non-coding, potentially regulatory regions.

Whole genome sequencing (WGS): The researchers have performed WGS analysis on 70 ASD subjects to investigate the role of rare genetic variants in this neurodevelopmental disorder. Whole genome sequence data for 20 subjects were obtained at the end of June 2013, and the analysis is still ongoing. Whole genome sequence for the initial set of 50 subjects (sequenced by July 2012) permitted: a) SNP discovery; b) development of computational tools for the annotation and the analysis of whole genome sequence; c) a comparison of rare variants in 50 ASD subjects with publically available datasets (1000 Genomes, exome data for 1500 ASD trios obtained from dbGAP).

Whole genome sequencing was performed on the Illumina HiSeq 2000 platform at the Penn Genomic Frontiers Institute (PGFI). Paired-end reads were mapped to the National Center for Biotechnology Information (NCBI) human reference genome (build 37.2). Variant calls were performed using the PGFI and DRAW pipelines. Median sequence coverage over all windows across the 50 samples was ~25x. Within each sample, averaged sequencing depth was >20x for

~70%. The ancestral allele was identified based on annotation from the 1000 Genomes Project and for novel variants the reference allele was used.

This analysis identified 11.1 million sequence variants within the 97% called genome fraction. The overall ratio of transitions (Ti) to transversions (Tv) was ~2.15 in each sample. From the ~3,79 million SNPs and indels detected in each individual, 22,000 fell into exonic regions, with an average of 15,000 coding or synonymous SNP variants and roughly 7,000 non-synonymous, frame shift or gain/loss of a stop-codon variants. The overall rate of novel SNP variants is ~3.4% with respect to dbSNP 132. Within the confines of this study, the team focused predominantly on SNP variants, as the reliability of the called genotypes is higher than for indels. The researchers initially searched for exonic missense variants common in the 40 individuals with ASD and rare (MAF < 2%) in both 1000 Genomes Project data and a set of 54 HapMap WGS made publicly available by Complete Genomics (http://www.completegenomics.com/public-data/). Variants that were observed in a single WGS were excluded. The resulting list of variants, labeled as "damaging/deleterious" by both Polyphen2 and SIFT, did not contain a sequence variant present in all ASD samples and absent or rare in the 1000 Genomes data, supporting previous reports on genetic heterogeneity.

Exonic variants were further analyzed using three annotation tools, ANNOVAR, Polyphen2, and SIFT. Among 7,000 exonic missense mutations per subject, ~ 1,900 were selected as "deleterious" or "damaging", ~ 425 were also novel (not reported in dbSNP or 1000 Genomes), and ~ 225 were novel and predicted to be "deleterious" or "damaging". Among previously reported ASD-associated genes, the reserachers have detected novel variants in *CACNA1H, CACNA1C, MADCAM1, CDH22, HTR2A and BZRAP1*. With the completion of WGS of the additional 20 samples, the researchers will perform comparisons of allele frequency for exonic with the allele frequency in:

a) 1000 Genomes (1000 controls)
b) Exome Variant Server (5000 controls)
c) 1000 ASD child-parent trios exome data (Autism Sequencing Consortium –from the dbGAP)
d) 500 CAR ASD subjects genotyped using the Illumina Human Exome Beadchip (these genotype data were generated in late June and are currently being analyzed)

Analysis of non-coding regions: With the availability of WGS for 50 ASD subjects, the researchers examine variants in intergenic and intronic regions of 100 ASD associated genes. Specifically, the research team developed a computational approach that combines data on epigenetic marks to identify genomic sequences with a potential regulatory role in brain tissues. Specifically, the reseachers integrate information on DNase1 hypersensitivity and active histone modification marks (H3K4me3, H3K4me1 and H3K9ac) from the human fetal brain in a logistic regression model to estimate the Brain Regulatory Potential (BRP) genome-wide. The reseachers demonstrated that the BRP score is significantly effective in detecting experimentally validated enhancers, known disease causing regulatory mutations, as well as single nucleotide polymorphisms associated with expression quantitative trait loci (eQTL SNPs). The researchers performed the analysis of regulatory potential for intergenic and intronic regions of 100 ASD associated genes. For example, this analysis identified a novel SNP in three unrelated individuals within the promoter of the gene *CADM1* that resides not only within a BRP element.

but also within a site likely to be bound by transcription factors NRSF, FOXP2 and RXLCH (Figure 1). Other examples of rare SNPs as plausible candidates for regulatory variants include SNPs in genes such as *CDH10, MCPH1, AUTS2, REEP3,* and *PCDH10* (Wadhawan et al., in preparation*)*.

The role of developmental genes in ASD: Through the analysis of whole genome sequence for 50 ASD subjects, the researchers have developed several tools and databases that can be applied in the analysis of genetic variation in different gene sets and address the role of these genes to a specific disease. For example, the researchers performed extensive analyses of genes that are necessary for basic developmental function, so called essential genes (Georgi et al., 2013). The research team showed strong and consistent signatures of purifying selection within the set of essential genes, including increased sequence conservation, a reduced number of exonic missense variants, and an overall shift in allele frequency towards rare alleles. Leveraging these results, the reseachers then showed that *de novo* mutations in ASD cases are significantly enriched in this gene set in data from recent papers related to Autism Spectrum Disorders (ASD). Among the 259 essential genes with *de novo* events, 179 genes are hit by events exclusively in ASD cases (Figure 2). Nine out of the 179 essential genes are also ASD candidates (*PTEN, FOXP1, CHD7, MEF2C, STXBP1, TSC2, CASK, GRIN2B,* and *SCN1A*). These genes, as well as others from the essential gene network, will be our primary targets for genotype-phenotype correlations in ASD subjects, those recruited at the CAR and available in other collections.

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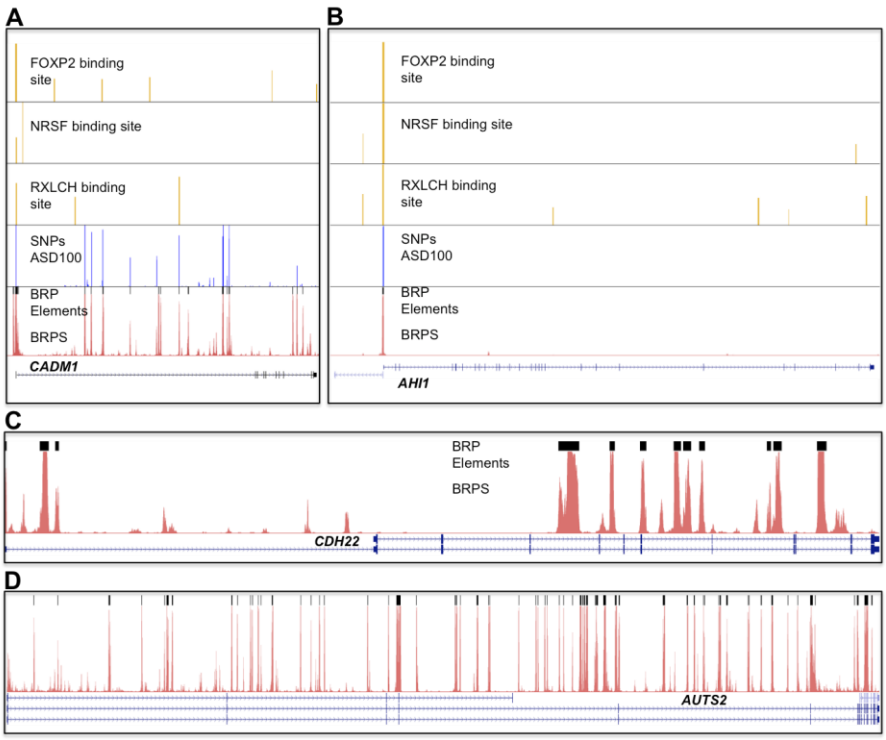

Figure 1: Application of BRP to known ASD-candidate loci. BRP was applied to annotate SNPs identified from resequencing of 100 ASD-candidate loci in 20 ASD individuals. Illustrated are two ASD-candidate loci (A) *CADM1* and (B) *AHI1* harboring SNPs within BRP elements that also overlap with putative TFBS corresponding to transcription factors NRSF, FOXP2 and RXLCH. Furthermore, BRP was used to identify cases of coding exons with the potential to act as regulatory sequences. Illustrated are two ASD-candidate loci (C) *CDH22* and (D) *AUTS2* containing coding exons that overlap with BRP elements.

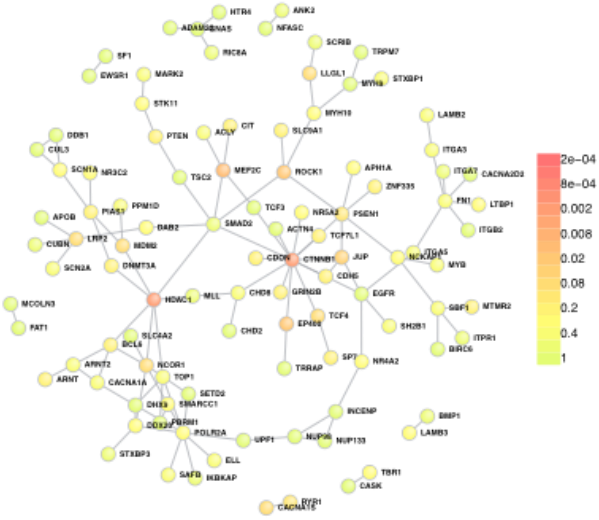

Figure 2: Protein-protein interaction network of 179 essential genes with de novo variants in ASD cases but not family-based controls generated by DAPPLE. Circles denote genes and lines connecting genes indicate physical interactions. Here, only direct connections among the 179 are plotted. The colors indicate the statistical evidence for the enrichment of connectivity for an individual gene in the given network. The significance of the overall excess of connectivity in this network is P=0.0019 (114 direct connections observed, ~84 expected).

PROJECT 3: ANIMAL MODELS OF GENETIC INFLUENCES ON BRAIN AND BEHAVIOR. (PI: EDWARD BRODKIN, MD; CO-PI, EDWIN ABEL, PhD)

Specific Aim 3.1. Define the role of a specific cadherin 9 gene (*Cdh9*)-cadherin 10 gene (*Cdh10*) intergenic region in endophenotypes of autism spectrum disorders (ASD) in an engineered deletion mouse.

The original goal for Specific Aim 3.1 was to generate a *Cdh9-Cdh10* intergenic region deletion so that, by Year 2, the researchers would have some live mice to begin breeding and phenotyping. Because the originally-proposed deletion of a 168 kilobase region is technically very difficult and risky, as pointed out by reviewers of the grant proposal and as realized by this research group, the team decided to generate a mouse in which the *Cdh10* gene itself is flanked with loxP sites and then conditionally deleted. This decision was discussed in the Progress

Report in the year 2010. The researchers contracted with a company, GenOway to generate a conditional knockout of *Cdh10*, as discussed in the Progress report of 2010. GenOway generated a mouse in which exons 4 and 5 of *Cdh10* are flanked with loxP sites and then conditionally deleted. This strategy results in the conditional deletion of 415 basepairs of coding sequence encoding the 5' part of the cadherin domain of Cdh10 protein. Furthermore, this deletion induces an out of frame splicing generating a premature Stop codon in exon 6b. As noted in the 2011 and 2012 progress reports, the process of GenOway generating these mice took much longer than anticipated, due to technical difficulties attributable to the GC-rich nature of the sequence around *Cdh10*. However, these technical challenges were ultimately surmounted, and, in Year 4 of the award, floxed *Cdh10* on a pure C57BL/6 genetic background arrived at University of Pennsylvania, and mice were bred at U Penn. Due to receiving the mice in the last year of the award, and the start up time required to breed a sufficient number of mice and cross them with a cre-expressing mouse line, there was time in the award period only to initiate testing of social behaviors in this line. The *Cdh10-flox* line was crossed with a *CaMKII R4 cre* line that expresses cre postnatally throughout the forebarin. The mice were then tested in the Social Choice Test (a.k.a. The Social Approach Test) in a 3-chambered apparatus. Although the researchers originally planned to test social approach behavior in the Social Choice Test at 30 days of age, because the researchers changed the mouse model in Specific Aim 1 to a conditional deletion model (see rational above), they decided to start phenotyping by assessing behaviors at ~8-weeks-of-age, when they were more confident that Cdh10 would be deleted in this model system.

Social choice test. Sociability of a "test" mouse (mutant or wildtype mouse) towards an unfamiliar gonadectomized A/J "stimulus" mouse was measured using a social choice task in a dimly lit (5 lux) testing room. The methods for this social choice task have been developed in the Brodkin lab, and employ a three-chambered apparatus (see **Figure 1**).(Brodkin et al., 2004, Sankoorikal et al., 2006, Brodkin, 2007, Fairless et al., 2008) Time spent in each chamber, time spent sniffing the cylinders, and numbers of transitions between the chambers were measured in **Phase 1** (10 min habituation period, no stimulus mouse present) and **Phase 2** (10 min social choice period, stimulus mouse in social cylinder; paperweight in nonsocial cylinder). Then, the cylinders were removed, and the test and stimulus mouse were allowed to interact freely for 5 minutes **(Phase 3)**, during which the test mouse was scored for time spent in social affiliative vs. aggressive vs. nonsocial/repetitive behaviors.(Miczek et al., 2001, Spencer et al., 2005)

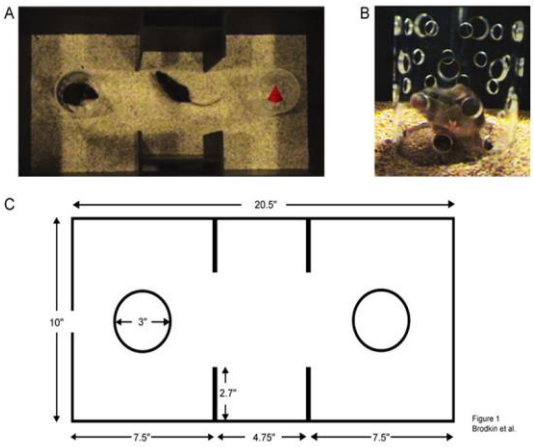

Figure 1. The Social Choice Test Apparatus. A. The 3-chambered apparatus is shown during Phase 2 of the test, with a stimulus mouse in the social cylinder and a novel object (paperweight) in the nonsocial cylinder. Light is used in the picture, but the test actually is carried out in complete darkness, and videotaped with a camera with infrared detection. **B.** A generic stimulus mouse is shown in the social cylinder, but in the experiments conducted, a gonadectomized A/J stimulus mouse was used in the nonsocial cylinder. **C.** Dimensions of the apparatus.

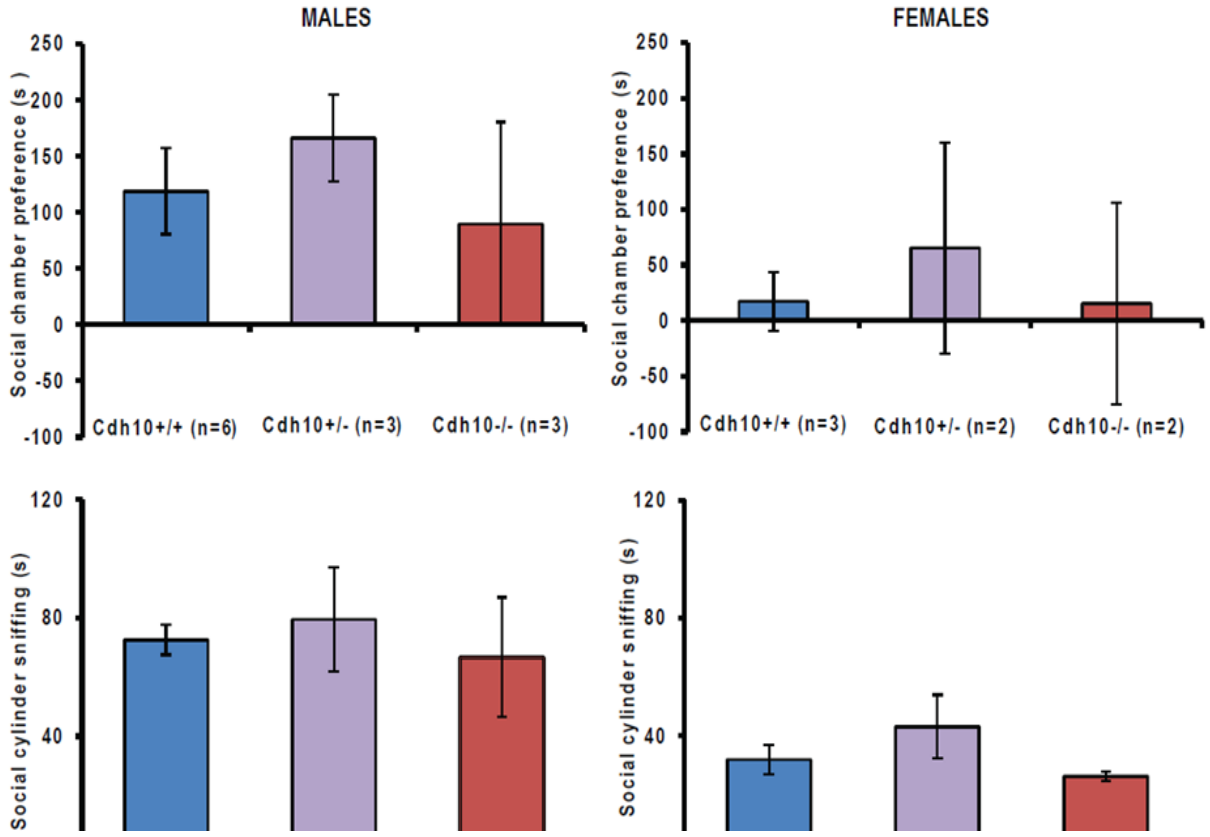

Figure 2: Social approach behavior in *Cdh10* conditional mutant animals in the Social Choice Test. The data presented are on 8-week-old male mice. Cdh10+/+ mice are either heterozygous or homozygous flox (+/flox or flox/flox), but had no cre (+/+). *Cdh*10+/- mice are heterozygous for flox (+/flox) and heterozygous for *cre* (+/*cre)*. *Cdh10*-/- mice are homozygous for flox (flox/flox) and heterozygous for cre (+/*cre*). Social chamber preference = (time in the social chamber – time in the nonsocial chamber during Phase 2) - (time in the social chamber – time in the nonsocial chamber during Phase 1). Social cylinder sniffing = time that the test mouse spent directly sniffing the social cylinder in Phase 2. A. Males: by ANOVA or student's t-tests, there were no significant differences in Social Chamber Preference or in Social Cylinder Sniffing among the genotypes. B. Females: there were no significant differences in Social Chamber Preference or in Social Cylinder Sniffing among the genotypes.

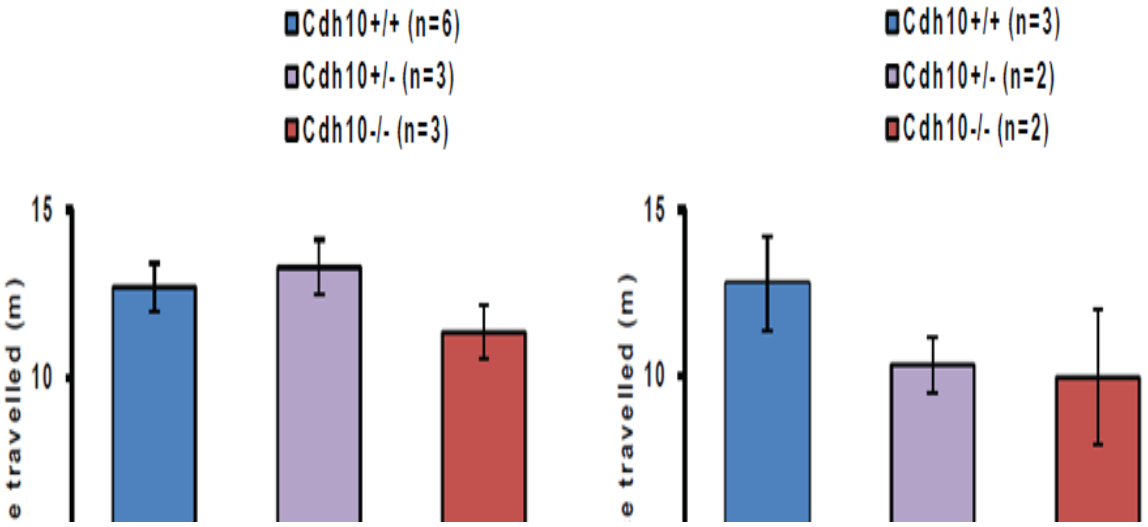

Figure 3. Locomotor activity of *Cdh10* conditional mutant animals in the context of the Social Choice Test. There was no difference among the genotypes in baseline locomotor activity in the context of the Social Choice Test, specifically in distance travelled by the test mice during the Phase 1 of The Social Choice Test.

Generation of the *Cdh9* conditional knockout mouse using the cre-lox system was initiated during Year 2 by the Knockout Mouse Project (University of California Davis Mouse Biology Program). The project was able to successfully produce homologous recombination for the *Cdh9* locus in embryonic stem cells. However, repeated rounds of blastocyst injections of clones only produced a low percentage of chimeras, and only one injection attempt successfully produced chimeras. However, chimera mice from this line only produced wildtype pups, i.e. germline transmission of the conditional knockout allele was not achieved, despite multiple attempts to do so, extending into the last year of the award.

Specific Aim 3.2. Define the role of the protocadherin 10 gene (*Pcdh10*) in endophenotypes of ASD in studies of a *Pcdh10* knockout mouse. The original goal for Specific Aim 2 in Year 1 was to obtain constitutive heterozygous *Pcdh10* knockout mice (*Pcdh10*$^{+/-}$ mice), and to begin breeding and phenotyping of these mice at University of Pennsylvania. Rather than obtaining these mutant mice on a mixed C57BL/6 (B6) -129 genetic background from the Texas Institute for Genomic Medicine (TIGM), as originally proposed, the researchers obtained the same mutant mice live on a pure B6 genetic background from a collaborator, Dr. Shinji Hirano, at Kochi University in Japan. The study of these mice on a pure genetic background had significant advantages in minimizing potential confounding genetic variability among the mice. Also, because the researchers were able to obtain the mice live, we maximized the chances of our proceeding to experiments quickly. The deletion of *Pcdh10* was made by replacing the whole first exon of *Pcdh10* with a *lacZ-neo* selection cassette.(Uemura et al., 2007) The mice were backcrossed to C57BL/6 for more than 15 generations in the Hirano lab, and, since arrival in the Brodkin lab, underwent 5 additional generations of backcrossing to the C57BL/6J strain. The mouse line arrived in the Brodkin lab in Year 1 of the award, but, by the time they went through quarantine, sufficient numbers were not generated to begin behavioral phenotyping until the end of Year 1 of the award. The mouse line was maintained by breeding male *Pcdh10*$^{+/-}$ mice with

female C57BL/6J mice. The sample size of the various behavior experiments were typically less than 20 mice per subgroup that was anticipated in the original application. There were several reasons for this: 1) the ratio of *Pcdh10*$^{+/-}$ pups to WT pups was less than 50%, which reduced our ability to generate sample sizes, 2) the researchers decided to use more separate cohorts than proposed in the original application in order to carry out the many behavioral tests, because we were concerned about the potentially confounding effects of earlier behavioral tests on later behavioral tests; and 3) sample sizes substantially smaller than 20 are typically used in our lab and in the general literature on mouse behavioral phenotyping.

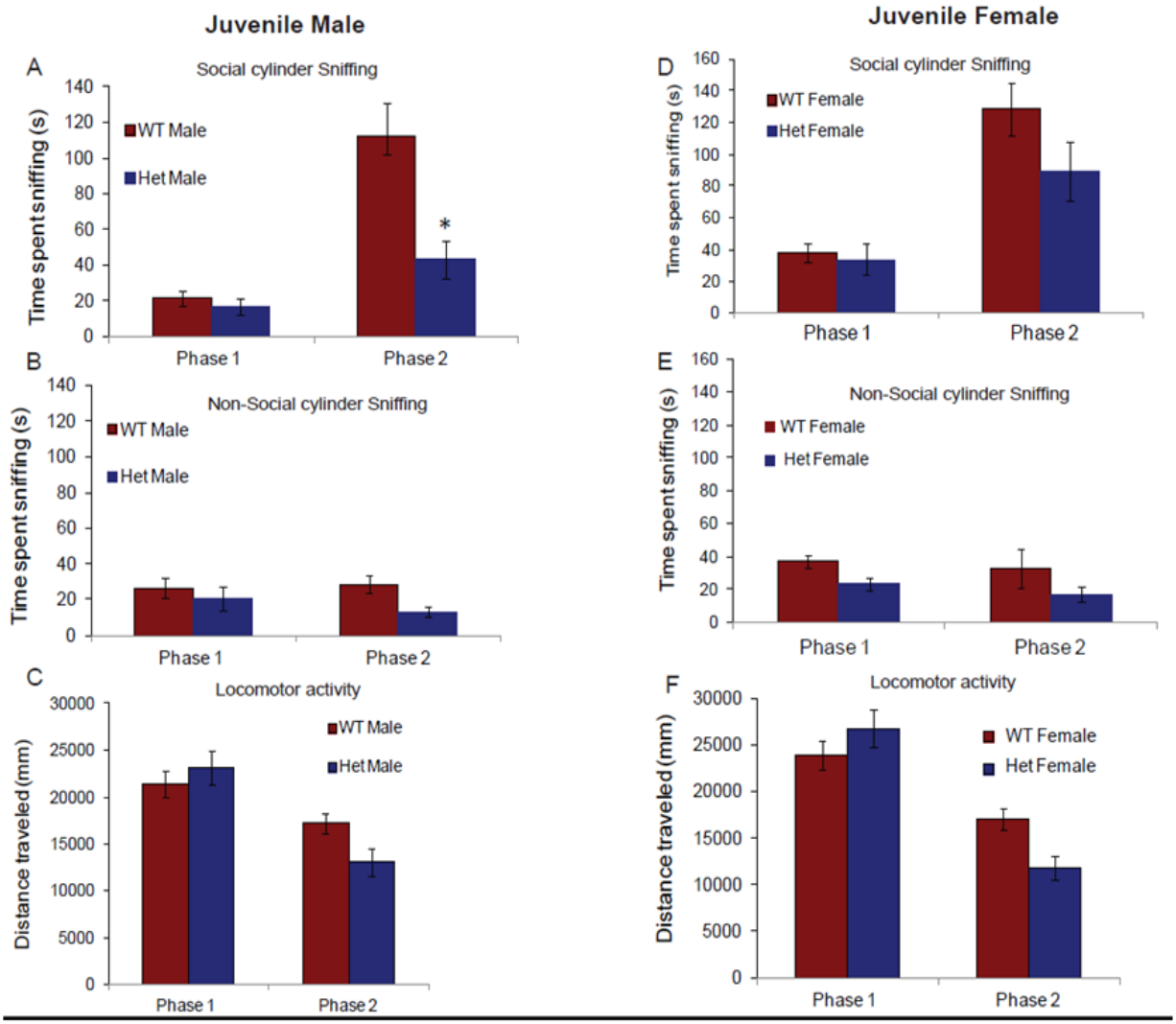

Figure 4. **Reduced sociability in juvenile male *Pcdh10*$^{+/-}$ mice (heterozygous, HET) relative to male wildtype (WT) littermates, but no significant difference in sociability in female mice.** **A.** During Phase 2, male *Pcdh10*$^{+/-}$ mice (n=13) show significantly reduced time sniffing the social cylinder relative to wildtype (WT) littermates (n= 15) mice (rmANOVA Main effect of genotype $F(1,26)=8.33$ $p<0.01$; rmANOVA, genotype x phase interaction $F(1,26) = 10.46$, $p<0.005$). Thus, juvenile male *Pcdh10*$^{+/-}$ mice show reduced social approach and investigation behaviors relative to male wildtype (WT) littermates. **B.** There was no significant difference in

non-social cylinder sniffing in Phase 1 or Phase 2 in male *Pcdh10*$^{+/-}$ mice relative to male wildtype (WT) littermates. Therefore, the reduction in sniffing behavior (see A above) appears to be specific to social stimuli, and does not generalize to novel, non-social stimuli. **C.** There was no significant difference in locomotor activity between juvenile male *Pcdh10*$^{+/-}$ mice relative to wildtype (WT) littermates during either Phase 1 or Phase 2 **D.** There was no significant difference in time sniffing the social cylinder in juvenile female *Pcdh10*$^{+/-}$ mice (n=12) relative to WT littermates (n= 13). **E.** There was no significant difference in non-social cylinder sniffing during either Phase 1 or Phase 2 in juvenile female *Pcdh10*$^{+/-}$ mice relative to WT littermates. **F.** No significant difference in locomotor activity during either Phase 1 or Phase 2 in juvenile female *Pcdh10*$^{+/-}$ mice relative to WT littermates.

Using the Social Choice Test in a 3-chambered apparatus (see description above), the researchers identified a highly significant reduction in sociability specifically in male (but not in female), juvenile (~30-day-old) heterozygous protocadherin gene deletion mice (*Pcdh10*$^{+/-}$ mice) relative to wildtype littermates (WT). This reduced sociability of male *Pcdh10*$^{+/-}$ mice was not attributable to a confounding alternation in locomotor activity, as there was no difference in locomotor activity in *Pcdh10*$^{+/-}$ mice relative to WT in the context of the Social Choice Test.

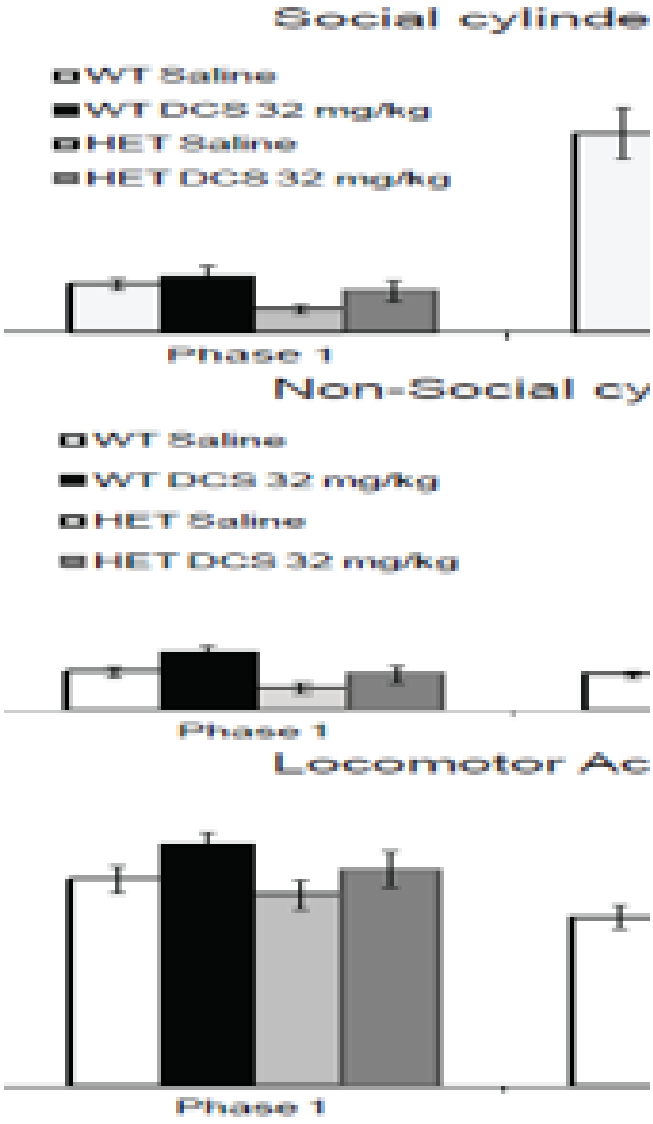

Figure 5. Acute d-cycloserine (DCS) treatment rescues the sociability deficit in juvenile male *Pcdh10*$^{+/-}$ mice. A. Social cylinder sniffing in Phase 2 of the Social Choice test after acute d-cycloserine (DCS, 32mg/kg) treatment **i**n juvenile male *Pcdh10*$^{+/-}$ mice (heterozygous, HET) and male wildtype (WT) littermates. Both HET and WT mice show a significant increase in sniffing time when the social and non-social stimuli are introduced during phase 2, when compared with phase 1 (rmANOVA, main effect of phase $F(1,34= 186.2, p<0.0001$). During Phase 2, acute DCS treatment increases social cylinder sniffing in HET mice (saline n= 9 vs. DCS treatment n=9, Bonferroni Dunn post hoc p=0.01) but does not alter sniffing time in WT mice (saline n=11 vs. DCS n=9 treatment, p>0.05 not significant). It is important to note that, in this separate cohort of mice, the researchers replicated the reduced sociability of *Pcdh10*$^{+/-}$ mice in the context of the social choice test (as shown above in Figure 4), demonstrating that this phenotype is robust. **B.** Non-social Cylinder sniffing in juvenile male WT and HET mice. There is no significant increase in non-social cylinder sniffing during

Phase 2 in juvenile WT male or Het male mice. (rmANOVA, no significant effect of phase $F(1,34)=0.50$ $p>0.05$). **C.** Locomotor Activity during social choice test in WT and HET mice. There is a significant decrease in locomotor activity after exposure to the social and nonsocial stimuli in Phase 2 in both WT and HET mice (rmANOVA, significant main effect of phase, $F(1,34)=82.9$, $p<0.001$). However, there is no significant difference in locomotor activity between juvenile male WT or HET mice treated with saline or DCS 32mg/kg (rmANOVA, (main effect of genotype $F(1,34)=6.5$ $p=0.02$, no significant interaction genotype*treatment $(F1,34)=1.3$, $p>0.05$); no significant effect Bonferroni post hoc $p>0.05$) during Phase 1 or Phase 2.

As an additional experiment added to those originally proposed, the researchers sought to determine whether they could pharmacologically rescue the reduced sociability of male *Pcdh10*$^{+/-}$ mice. The researchers felt that this experiment would have an important translational relevance to human ASD. Because there is evidence that the *Pcdh10* gene is involved in development, pruning, and function of glutamatergic synapses in the postnatal brain (Tsai et al., 2012), the research team hypothesized that a drug that augments glutamatergic signaling, specifically NMDA receptor signaling, such as d-cycloserine (DCS) at a moderate dose (32 mg/kg administered systemically), might rescue sociability in the male *Pcdh10*$^{+/-}$ mice. Also, there is some published evidence that DCS may augment sociability in humans with ASD (Posey and al., 2004). The researchers carried this pharmacologic experiment out in a separate cohort of juvenile (~30-day-old) male *Pcdh10*$^{+/-}$ mice and WT littermates.

Although the researchers observed reduced sociability of juvenile male *Pcdh10*$^{+/-}$ mice in the Social Choice Test, we did not observe a reduction in home cage social behavior among *Pcdh10*$^{+/-}$ mice and WT littermates housed together. In this test of home cages social behaviors, interactions between two mice of the same genotype and sex, housed together, were videotaped in their home cage. Each pup was observed at 60 second intervals evenly distributed through the 30 minute video, and the number of times that each mouse engaged in one of various social or non-social behaviors was counted. The methods used have been reported previously by our research group (Fairless et al., 2012).

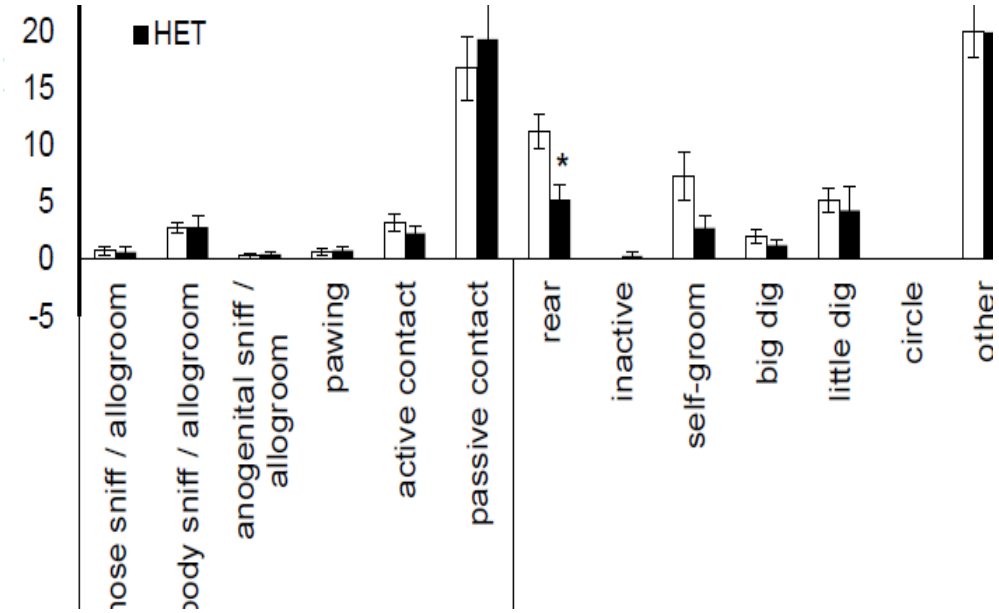

Figure 6. No significant difference between juvenile male *Pcdh10*$^{+/-}$ mice and WT mice in home cage social behaviors. There were no significant differences between male *Pcdh10*$^{+/-}$ mice (n=7) and WT mice (n=8) from 4 separate litters in home cage social behaviors. There was a difference in rearing behavior between juvenile male WT and *Pcdh10*$^{+/-}$ mice at the level of $p<0.05$ (Student's t test $p=0.02$), however this difference did not survive Bonferroni correction of the significance threshold. Thus, the reduced sociability toward unfamiliar stimulus mice in the Social Choice Test in male *Pcdh10*$^{+/-}$ mice was not seen in their interactions with familiar littermates in the home cage environment. The hypothesis for why the researchers found sociability deficits in the Social Choice Test (Figures 4 and 5 above), but not in the home cage test is that the two behaviors are likely to have somewhat different underlying neural circuitry and mechanisms involved. For example, our data (see Table 2 and Figure 16 below) indicate that the basolateral amygdala is activated during the Social Choice Test, but it is less likely to be activated during home cage social interactions, though the research team did not study brain region activation during home cage interactions. It is noteworthy that Pcdh10 is expressed at high levels in the basolateral amygdala in postnatal mouse brain (Aoki et al., 2003).

The researchers also compared social memory in juvenile male *Pcdh10*$^{+/-}$ mice vs. WT littermates. The research team found no significant difference between the genotypes in habituation (a tendency to sniff less) with repeated presentations of the same stimulus mouse, or in dishabituation (a tendency to sniff more) with presentation of a novel stimulus mouse. The stimulus mice used were gonadectomized A/J mice of the same sex at the test mice.

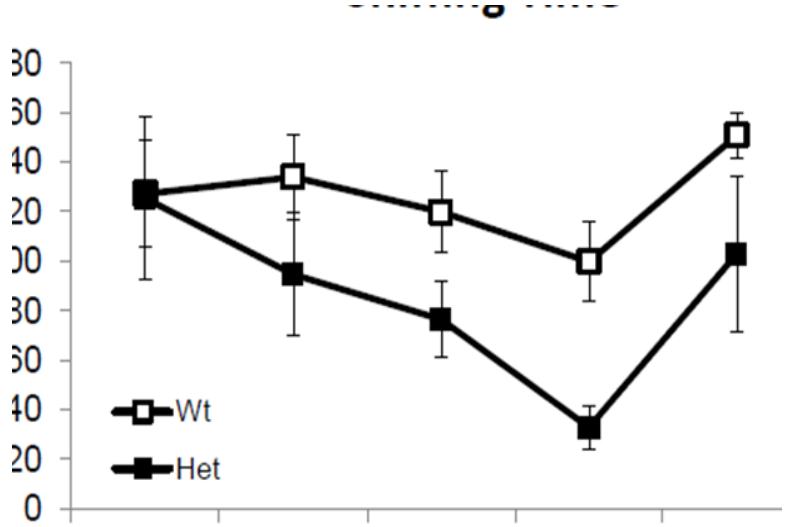

Figure 7. No significant difference between juvenile male *Pcdh10*$^{+/-}$ mice and WT mice in social memory. Both juvenile male WT (n=6) and HET (n=6) mice show a significant decrease in time spent sniffing the same stimulus mouse over the 4 trials (rmANOVA F 3,36= 5.07, p= 0.006). In addition, both WT and HET mice show an increase in time spent sniffing the novel mouse when compared with the time spent sniffing at the last presentation of the similar mouse (rmANOVA F 1, 12= 17.29, p=0.002), thereby revealing that both juvenile WT and HET mice have intact social memory.

To determine whether the reduced sociability of juvenile male *Pcdh10*$^{+/-}$ mice as observed in the Social Choice Test was attributable to any deficit in olfactory function, generalized anxiety-related behaviors, or balance and coordination, or to determine whether there was any defect in these behaviors in female *Pcdh10*$^{+/-}$ mice, the researchers carried out a series of tests in a separate cohort of juvenile male and female *Pcdh10*$^{+/-}$ mice and WT littermates. In addition to using the latency to find buried food test (see further below), we added the olfactory habituation-dishabituation test, which assesses olfactory acuity and discrimination to both social and nonsocial odorants (Yang and Crawley, 2009). In the olfactory habituation-dishabituation test, a mouse is presented with 3 consecutive exposures of the same odorant on a cotton swab, followed by 3 consecutive exposures of a different odorant, and so on. In the test, the researchers used both nonsocial odorants (swabs of water, almond, vanilla) and social odorants (swabs of soiled bedding from same sex and opposite sex mice). The typical pattern observed in a mouse that is able to detect odorants and discriminate between different odorants is habituation to repeated presentations of the same odorant (reduced sniffing time upon repeated presentations) and then dishabituation upon presentation of a new odorant (an increase in sniffing time upon presentation of a new odorant). Procedures used to carry out the elevated zero maze test of anxiety-related behavior and the accelerating rotarod test of balance and motor coordination are standard and were described in the original application. The researchers found no differences between *Pcdh10*$^{+/-}$ mice and WT littermates in any of these other behaviors.

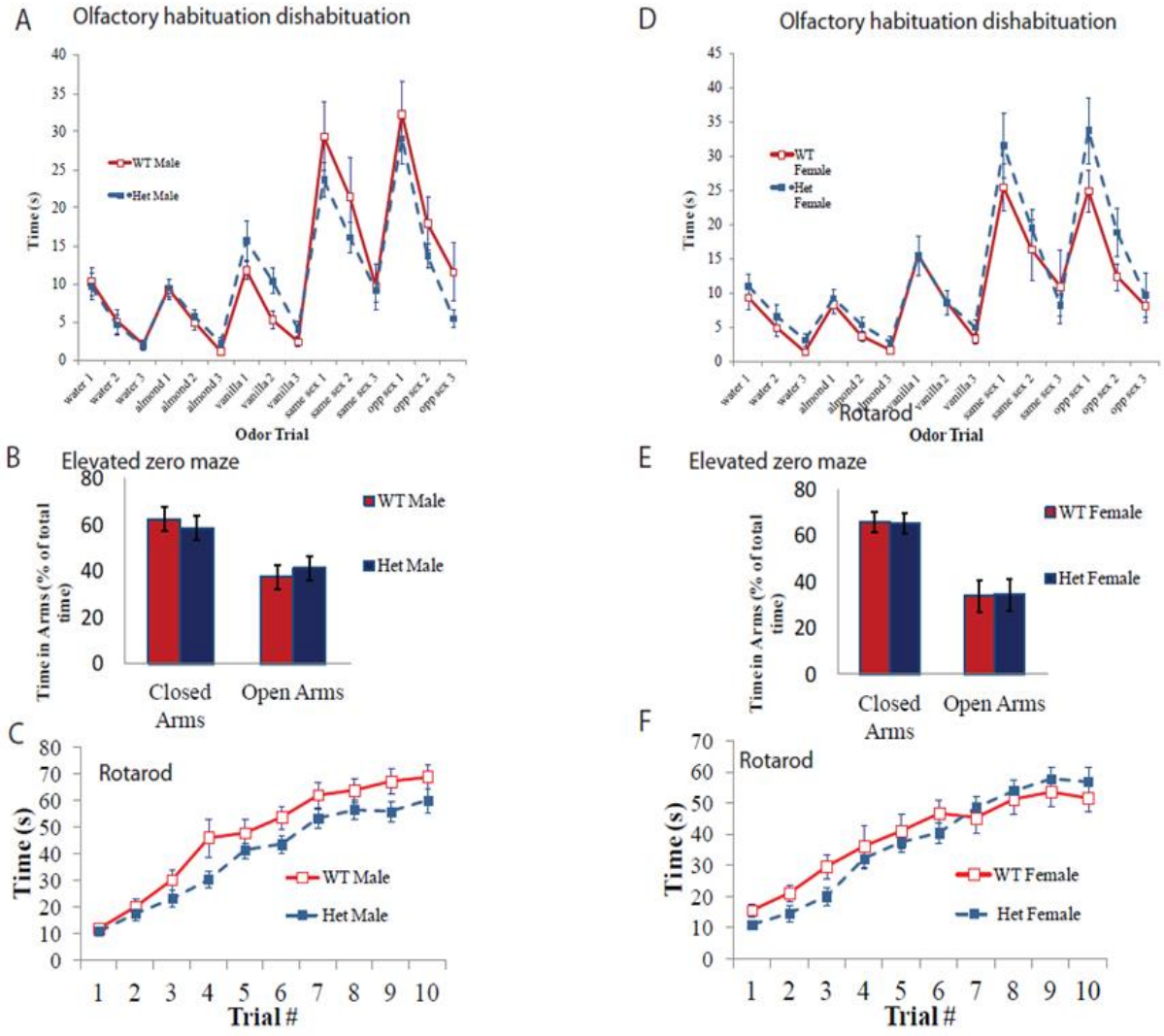

Figure 8. Juvenile male and female mice show normal olfactory, anxiety-like and locomotor activity. **A.** Olfactory habituation dishabituation in juvenile male *Pcdh10*$^{+/-}$ (heterozygous, HET) and WT mice. Juvenile male *Pcdh10*$^{+/-}$ mice (n=13) mice do not show a significant difference in sniffing times when compared with the WT (n=13) mice (rmANOVA no main effect of trial or genotype). **B.** Elevated Zero maze. There is no significant difference in the percent of time juvenile male WT (n=13) or *Pcdh10*$^{+/-}$ mice (n=13) spent in closed or open arms in the elevated zero maze (one-way ANOVA (1,24)=0.275 p>0.05 not significant). **C**. Rotarod Activity. While both WT and *Pcdh10*$^{+/-}$ mice increase their time on the rotarod with repeated trials, there is no significant difference between the genotypes (no main effect of genotype rm ANOVA F(1,24)=3.4 p>0.05)). **D.** Olfactory habituation dishabituation in juvenile female *Pcdh10*$^{+/-}$ (heterozygous, HET) and WT mice. Both juvenile female WT (n=9) and *Pcdh10*$^{+/-}$ mice (n=9) mice show an overall change in sniffing times (rmANOVA main effect of trial F(1,14 = 26.7, p<0.001); however, they do not show a significant difference between groups when exposed to the odor stimuli (rmANOVA Main effect of genotype F(1,14)=1.8 p>0.05 not significant). **E.** Elevated Zero maze. There is no significant difference between the genotypes in the percent of time spent in closed or open arms in the elevated zero maze (one-way ANOVA

(1,16)=0.003 p>0.05). **F.** Rotarod Activity. There is no significant difference between the genotypes in the rate of acquisition of the rotarod task (no main effect of genotype rm ANOVA F(1,16)=0.404 p>0.05)).

In a separate cohort of *Pcdh10*$^{+/-}$ mice and WT littermates, the researchers carried out a study of neurodevelopmental ontogeny and general home cage behavior, as well as general health and neurological reflexes. The researchers decided to carry out this neurodevelopmental study in separate cohort from those mice studied for olfaction, anxiety-related behaviors, and motor coordination, because the team wanted to prevent any potential confounding effects of the neurodevelopmental studies on later behaviors (e.g. on elevated zero maze behavior). The sample included both males and females, and *Pcdh10*$^{+/-}$ mice and WT littermate groups were balanced by sex. The study team found that by postnatal day (PND) 21, *Pcdh10*$^{+/-}$ mice had significantly lower bodyweight relative to WT littermates, but not differ significantly in body length.

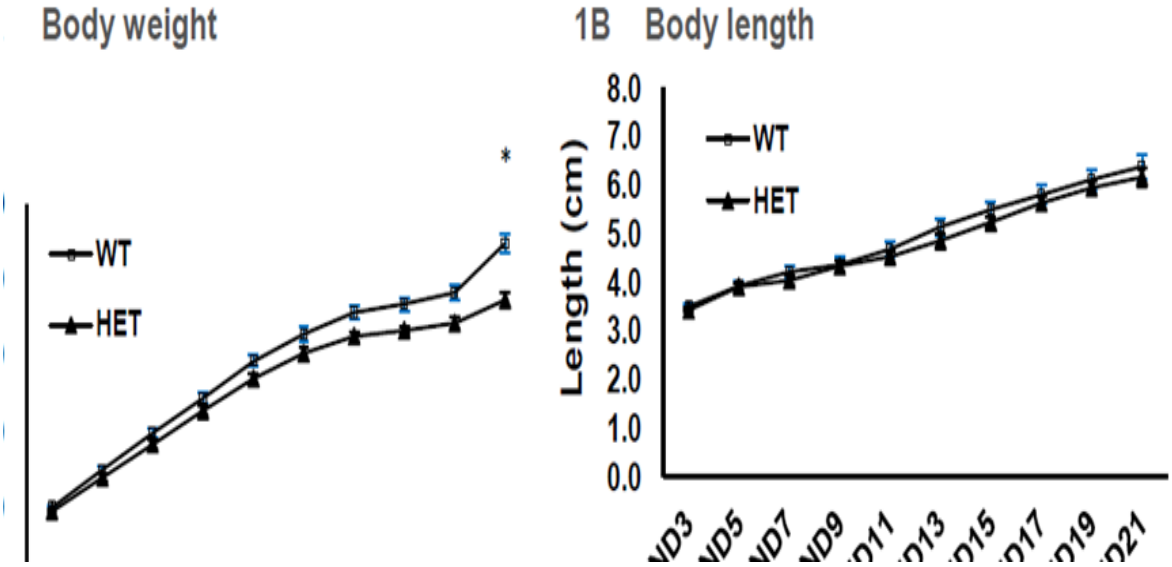

Figure 9. Growth in body weight and body length from postnatal day (PND) 3 to 21. 1A) There is a significant difference in overall body weight between WT (n= 35) and Pcdh10 HET (n=38) mice over development (rmANOVA, p <0.05), with HET mice gradually showing less weight gain over development. Post hoc analysis shows a significant difference in body weight at PND 21 (p<0.0001) between WT and HET mice. However, there was no overall difference in body length between WT and HET mice over PND 3- 21 (rmANOVA, p>0.05).

Table 1. Age of onset (in postnatal day) of sensory features and reflexes in WT and *Pcdh10*$^{+/-}$ mice.			
	WT	**HET**	**p value**
Whiskers present	3.1± 0.6	3.3 ± 1.1	p>0.05
Whisker orientation	8.1± 3.8	8.8 ± 4.0	p>0.05
Pinna detachment	5.7±1.3	5.4 ±1.3	p>0.05
Incisor appearance	8.6 ± 1.3	8.3 ±1.5	p>0.05
Forelimb reach	8.3 ± 2.8	8.4 ± 3.0	p>0.05
Hindlimb reach	10.5 ± 2.3	10.6 ± 2.3	p>0.05
Forelimb grasp	11.8 ± 1.6	11.9 ± 1.6	p>0.05
Eyes Open	14.3 ± 1.0	14.6 ± 0.8	p>0.05

Table 1 legend:
Screening of neurodevelopmental milestones revealed no significant differences in the age of onset of sensory features or reflexes in wild type (WT, n= 35) or Pcdh10 (HET, n=38) mice in 14 separate litters during postnatal development (PND 3-21) (p>0.05, rmANOVA).

Additionally, separate cohorts of *Pcdh10*$^{+/-}$ mice and WT littermates underwent a subset of behavioral tests in young adulthood, at approximately 2 months of age. The reason for this is that most of the literature on these behavioral tests (e.g. water maze, fear conditioning) is in adult mice, and therefore the investigators felt that the results would be more easily interpretable at an adult age. The first test in this older cohort was the open field test, with result shown below:

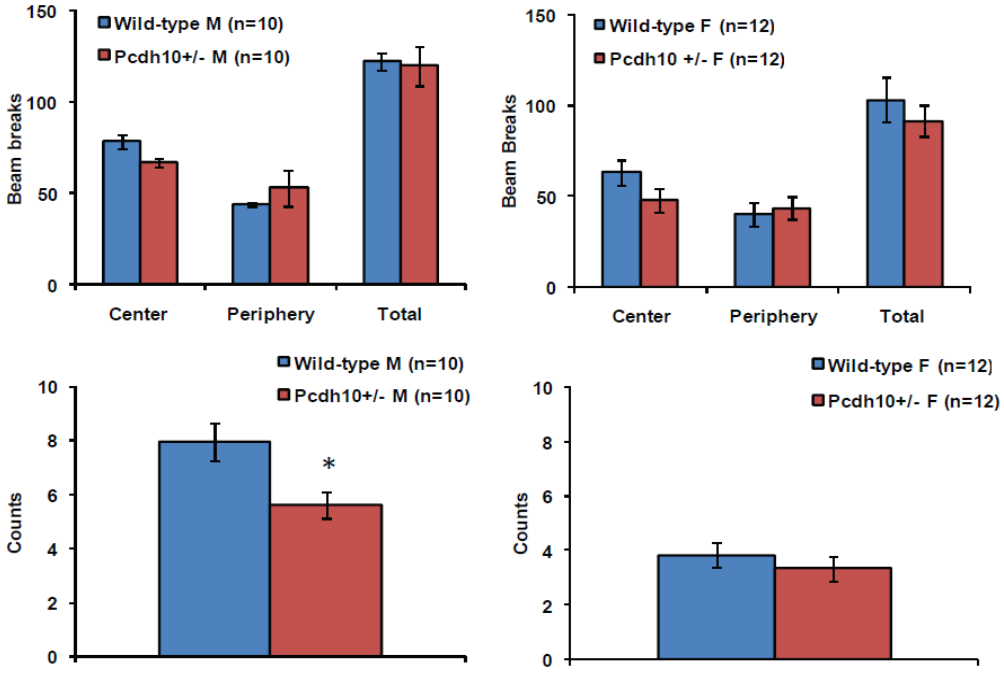

Figure 10. There were no differences in locomotor behavior in the Open Field Test in male or female *Pcdh10*$^{+/-}$ mice vs. same sex WT littermates. **A.** No difference in spontaneous locomotor behavior in the open field in male *Pcdh10*$^{+/-}$ mice. Student's t-test, center p<0.2,

periphery p<0.3, total p<0.6. **B.** No difference in locomotor behavior in *Pcdh10*$^{+/-}$ females. Student's t-test, center p<0.2, periphery p<0.9, total p<0.5. **C.** Male *Pcdh10*$^{+/-}$ mice have reduced rearing behavior in the open field. One-way ANOVA p<0.03. **D.** No difference in rearing in females One-way ANOVA, p<0.5.

In young adult mice, the investigators also carried out the rotarod test of balance and motor coordination.

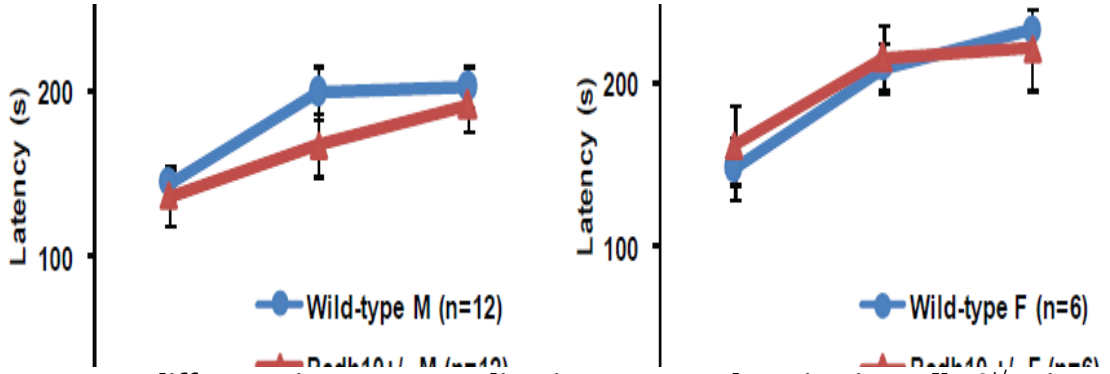

Figure 11. No difference in motor coordination or motor learning in *Pcdh10*$^{+/-}$ mice. Both male and female *Pcdh10*$^{+/-}$ mice perform and acquire the rotarod task at levels similar to that of wild-type siblings. ANOVA: Males genotype p<0.39, day p<0.001, genotype*day p<0.28; Females genotype p<0.9, day p<0.003.

We did find a male-specific deficit in long-term conditioned fear memory in *Pcdh10*$^{+/-}$ mice (recall that the sociability deficit in the Social Choice Test was also male specific).

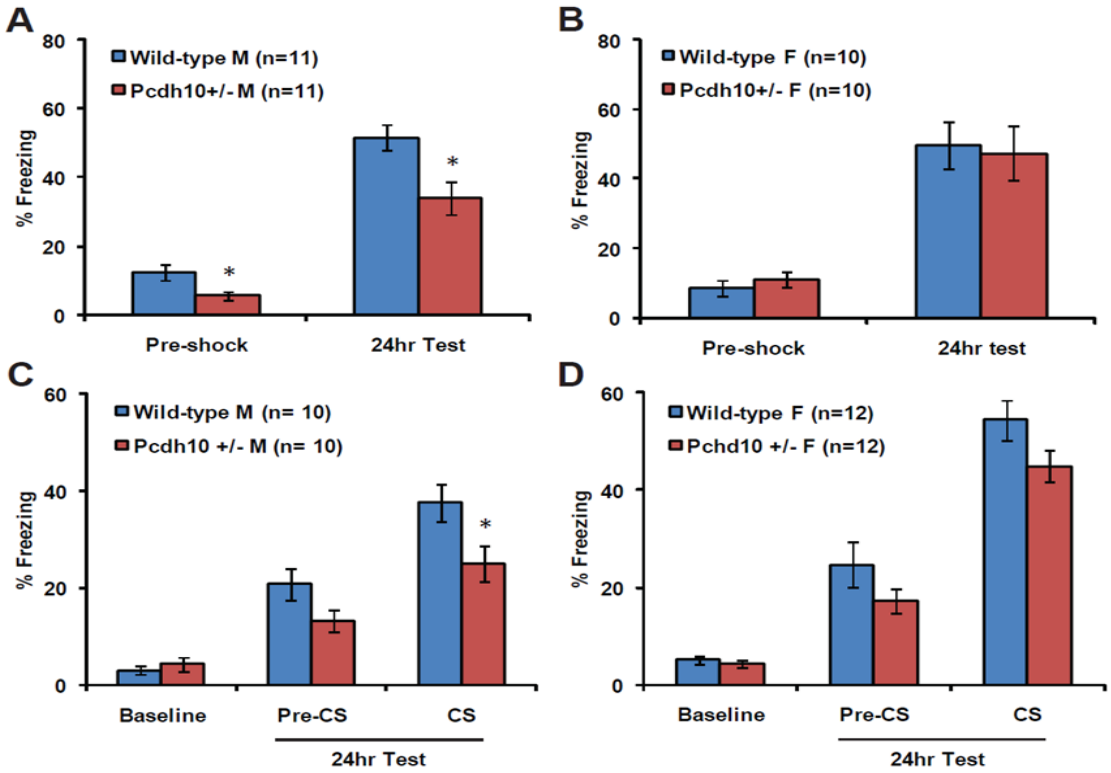

Figure 12. *Pcdh10*$^{+/-}$ mice exhibit male-specific deficits in long-term conditioned fear memory. A. *Pcdh10*$^{+/-}$ males exhibit reduced freezing when tested 24hrs after contextual fear conditioning. Student's t-test: Pre-shock $p<0.02$, 24hr test $p<0.02$. B. No difference in contextual conditioning in *Pcdh10*$^{+/-}$ females. Student's t-test: Pre-shock $p<0.5$, 24hr test $p<0.9$ C. *Pcdh10*$^{+/-}$ males show reduced freezing compared to wild-type littermates when tested for cued fear 24hrs after conditioning. Student's t-test, Base $p<0.6$, Pre-CS $p<0.08$, CS $p<0.02$. D. No difference in cued fear memory in *Pcdh10*$^{+/-}$ females. Student's t-test, Base $p<0.6$, Pre-CS $p<0.3$, CS $p<0.13$.

In addition to the behavioral tasks originally proposed, the study team assessed young adult *Pcdh10*$^{+/-}$ mice for another learning task, object recognition memory.

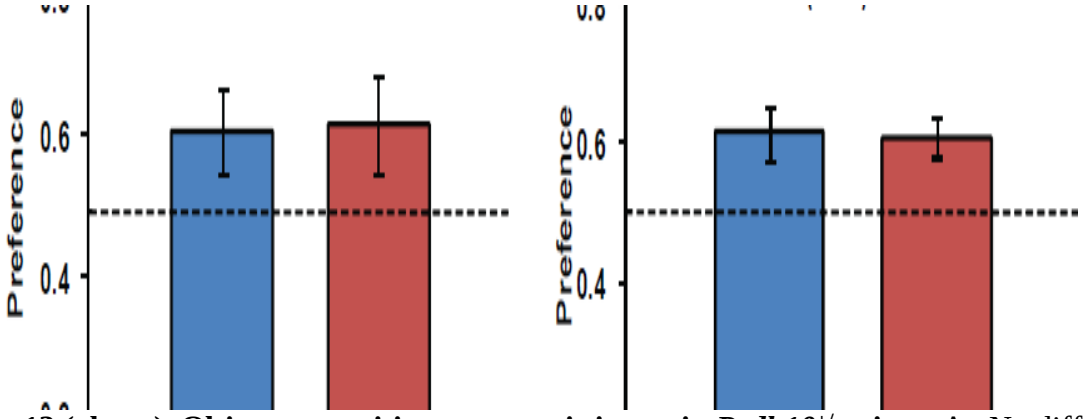

Figure 13 (above). Object recognition memory is intact in *Pcdh10*$^{+/-}$ mice. A. No difference in novel object recognition in male *Pcdh10*$^{+/-}$ mice when tested at 24hrs. Student's t-test, p<0.8 B. No difference in performance of female *Pcdh10*$^{+/-}$ mice in object recognition memory. Student's t-test p<0.93.

The researchers also assessed the mice in the Water Maze Task.

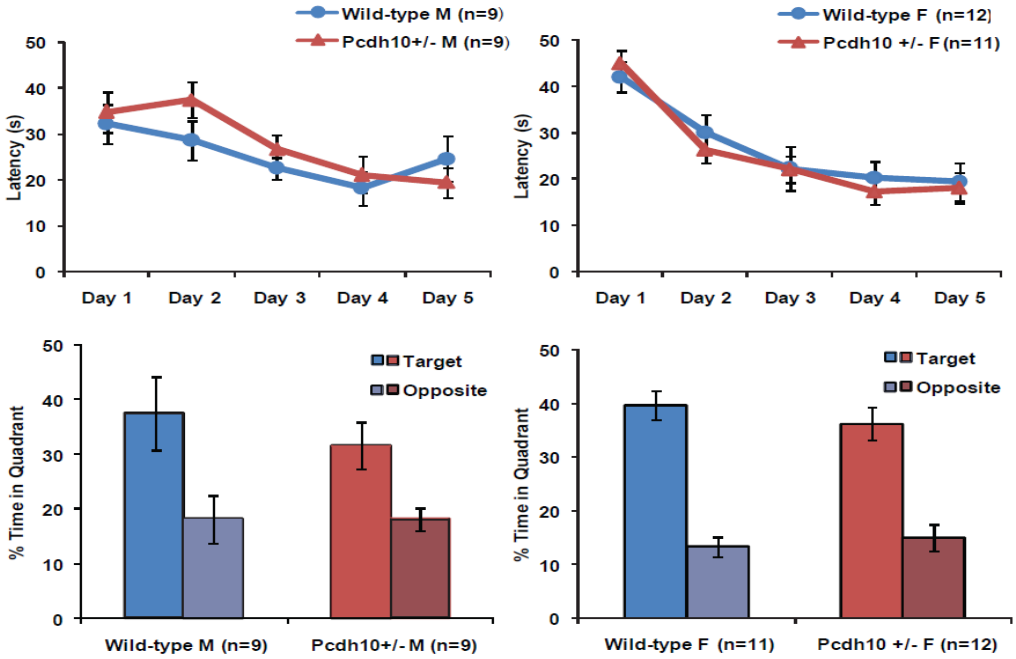

Figure 14 (above). No differences in spatial navigation in *Pcdh10*$^{+/-}$ mice in the Water Maze Task. No difference in acquisition of spatial version of the hidden platform water maze in *Pcdh10*$^{+/-}$ males (A) or females (B). C. After 5 days of training, both wild-type and *Pcdh10*$^{+/-}$ males showed a significant preference for the target quadrant over opposite quadrant in a probe trial. Student's t-test, wild-type p<0.03, Pcdh10+/- p<0.05. D. Females also showed significant preference for the target quadrant during the probe trial. ANOVA, genotype p<0.43, quadrant

p< 0.001, genotype*quadrant p<0.54.

Although the researchers found no difference between juvenile *Pcdh10*$^{+/-}$ mice and WT littermates in olfactory acuity and discrimination to social and nonsocial odorants in the olfactory habituation-dishabituation task (see Figure 8 above), the team found enhanced performance (reduced latency to find buried food) in adult *Pcdh10*$^{+/-}$ mice in the latency to find buried food task.

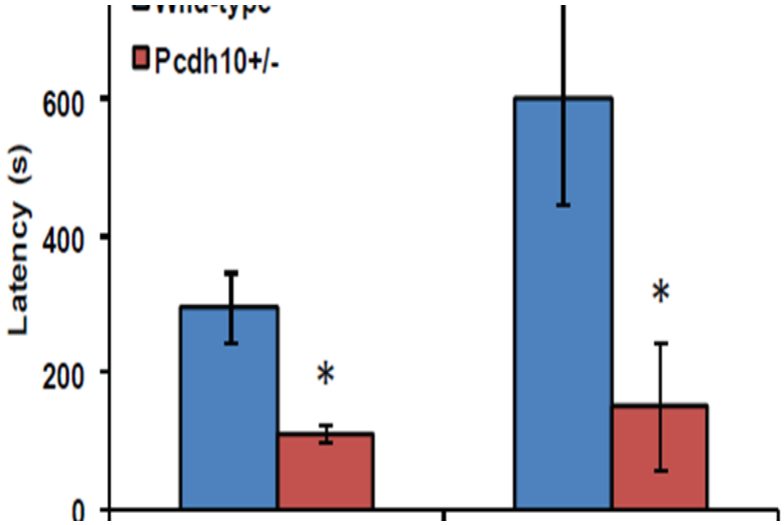

Figure 15. *Pcdh10*$^{+/-}$ mice exhibit enhanced performance in buried food task. Both male and female *Pcdh10*$^{+/-}$ mice show reduced latency to locate buried food. 2-way ANOVA, genotype p<0.01, sex p< 0.20, genotype*sex p<0.31.

In summary, the *Pcdh10*$^{+/-}$ mice showed male-specific deficits in sociability in the Social Choice Test and in long-term conditioned fear memory. Although the hippocampus is known to play an important role in long-term contextual fear memory, the neural circuitry that mediates behavior in the Social Choice test has not been well-defined. Therefore, the researchers reasoned that it would be important to determine brain regions activated by the Social Choice Task in the wildtype mice, in order to better direct studies of synaptic function. Therefore, the team used an immediate early gene study, specifically c-fos immunohistochemistry, to delineate brain regions activated by the social stimulus in the Social Choice Task in juvenile, male C57BL/6 mice, the genetic background of the *Pcdh10*$^{+/-}$ mice. The investigators did not have sufficient time and resources to study c-fos expression in the mutant animals as well, since this experiment was added on to the original aims, and was quite time and labor intensive. Our c-fos immunohistochemistry data were submitted for publication (Kreibich et al, submitted).

In this c-fos immunohistochemistry experiment, the test mice were habituated to the empty 3-chambered apparatus (Phase 1), and then exposed to a non-social stimulus in one cylinder and either a stimulus mouse (A/J) in the social stimulus (SS) cylinder for the Social Exposure (SOC) group, or an empty (EMP) cylinder for the Non Social Exposure (NS) group (Phase 2). The Home Cage (HC) group remained in cages in the testing room.

One hour post-Social Choice Test, mice were trans-cardially perfused with 4% paraformaldehyde. The brains were post-fixed for 24 hrs and cryoprotected in a 30% sucrose solution. Coronal cryosections (40 μm) were incubated with a rabbit polyclonal anti-c-Fos (1:2000 Santa Cruz Biotechnology, Santa Cruz, CA) antibody for 48 hours at 4^{o}C and then with secondary biotinylated goat anti-Rabbit IgG (1:200 Jackson Immunoresearch) antibody. The c-Fos immunoreactivity was visualized using standard ABC method (Vectastain ABC kit, Vector) and di-aminobenzidine (DAB)-nickel solution (Sigma Aldrich, St. Louis, MO). The average number of c-Fos+ cells (Counts (#)) in each of the 18 brain regions of interest was quantified in 3 separate cryosections using NIH Image J.For double immunofluorescence experiments, cryosections were incubated with a mixture of rabbit polyclonal anti-c-Fos antibody (EMD Millipore, 1:5000, Billerica, MA) and either a mouse anti-calcium calmodulin kinase II (CaMKII, 1:250; EMD Millipore) or anti-parvalbumin antibody (PV 1:250; Sigma Aldrich, St Louis, MO). A mixture of fluorescent secondary donkey anti-rabbit and goat anti-mouse antibodies (Alexa 488, Alexa 546, 1:200; Life Technologies, Grand Island, NY) were used to visualize staining. Three separate confocal (Leica SP5 AOBS) fluorescent pictures (10μm depth; green (c-Fos), red (CamKII or PV) and combination) of the BLA were generated (Leica and ImagePro software). The number of c-Fos+ and double-labeled nuclei were counted (NIH ImageJ, RG2B colocalization plugin) and the percent of co-labeled c-Fos+ cells was determined.

Based on human and rodent studies indicating its role in processing social stimuli, we hypothesized that the amygdala would be activated by social exposure in C57BL/6 (B6) mice. The researchers also hypothesized that there would be activation of other limbic regions implicated in social and emotional behaviors. Initially, the study team compared c-Fos+ counts among HC, NS, and SOC groups in 18 regions, including 16 limbic brain regions, as well as olfactory and accessory olfactory bulbs (**Table 2**). Twelve regions showed a significant change ($p < 0.05$ ANOVA HC vs. NS vs. SOC), including the basolateral amygdala (BLA) ($F(2,11)=7.31$, $p=0.009$) and the medial amygdala (MeA) ($F(2,11)=15.38$, $p=0.001$) but not the central amygdala (CEA) ($F(2,11)=1.60$ $p>0.05$)(Fig 1D-F) in B6 mice. Other regions that showed a significant difference included the cingulate cortex (CC), subiculum, paraventricular nucleus of the hypothalamus (PVN), nucleus accumbens (NAc), locus ceruleus (LC), infralimbic and entorhinal cortices, accessory olfactory bulb, CA3, and dentate gyrus (DG). Posthoc analyses revealed that all 12 regions showed increased c-Fos activation in NS vs. HC, but only the BLA showed more c-Fos activation in SOC vs. NS ($p=0.035$). Thus, while a number of regions were activated by placement in and exposure to the SAT testing apparatus, the only brain region that was specifically activated in response to social stimulus in the B6 mice was the BLA.

Table 2: Comparison of Fos expression over multiple brain regions in C57BL/6J (B6) mice exposed to SOC, NS and HC conditions. Bold means overall ANOVA p<0.05. *NS vs. SOC, †HC vs.NS, ^HC vs.SOC posthoc p<0.05).

		C57BL/6J		
rain region	**Bregma**	**Home cage**	**Non Social**	**Social**
lala-Basolateral*	-1.22 to -1.7	**27±14†**	**66±24**	**157±33**
ygdala-Medial	-1.22 to -1.7	16±4†^	48±3	51±7
ygdala-Central	-1.22 to -1.7	8±3	13±2	14±2
eus Accumbens	1.42 to -0.86	43±14†^	109±17	100±1
l Tegmental Area	-4.36 to -4.6	12±2	7±2	14 ±2
Accessory lfactory Bulb	2.96-2.46	41±7†^	126±26	109±1
lfactory Bulb	3.56	1308±417†	3002±439	2674±4
leus Stria Terminalis	0.26 to 0.14	24±2.9	48.3±11.5	50±9.3
pocampus-CA1	-1.34 to -2.06	31±8	58±11	52±10
pocampus-CA3	-1.34 to -2.06	20±5†^	48±6	44±3
pocampus-DG	-1.34 to -2.06	34±4	53±5	59±3
teral Septum	0.98 to 0.38	266±86	628±127	651±14
cus Ceruleus	-5.34 to -5.68	8±2†^	20±5	24±5
r Nucleus Hypothalamus	-0.34 to -0.58	38±10†^	89±12	82±6
Subiculum	-4.16 to -4.36	71±18†^	468±75	329±6
gulate Cortex	0.26 to -0.22	225±48†^	673±11	610±9
alimbic Cortex	1.94 to 1.34	30±5†^	77±7	77±11
orhinal cortex	-4.16 to -4.38	60±24†^	314±32	269±2

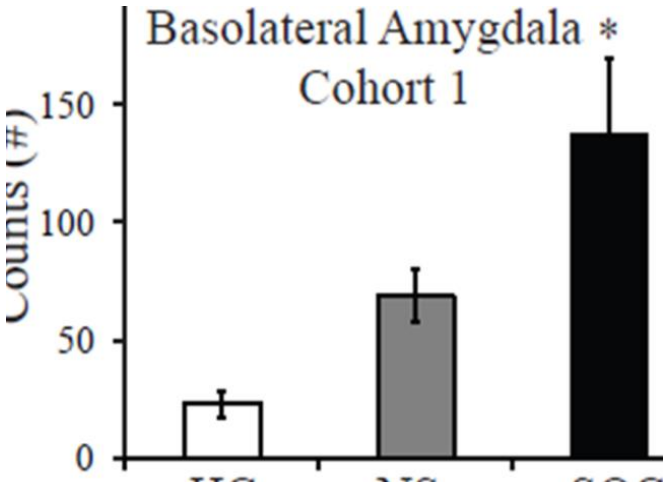

Figure 16. **Fos immunostaining** showing the number of c-Fos+ cells in the *(D)* BLA, *(E)* MeA and *(F)* CeA of B6 mice *(cohort 1)* exposed to home-cage (HC, n=4), NS (n=5) or SOC (n=5) conditions.
This results was replicated in two separate cohorts of C57BL/6J mice (see copy of Kreibich et al, submitted, included with this report).

Given these data on the activation of the amygdala in the Social Choice Task, as well as the known activation of the hippocampus in conditioned fear memory, the researchers decided to focus experiments on synapses on the hippocampus and amygdala, and the team also included the prefrontal cortex and nucleus accumbens, given their known role in motivation as well as social and emotional behaviors. Given the role of Pcdh10 in glutamatergic synapses (Tsai et al., 2012), the investigators examined the expression of a number of glutamate receptors in the 4 brain regions in in *Pcdh10*$^{+/-}$ mice vs. wildtype littermates. In order to maximize the efficiency and quantitative nature of the experiment, the research team used qPCR to compare expression of these receptor genes, rather than immunohistochemistry or immunoblotting.

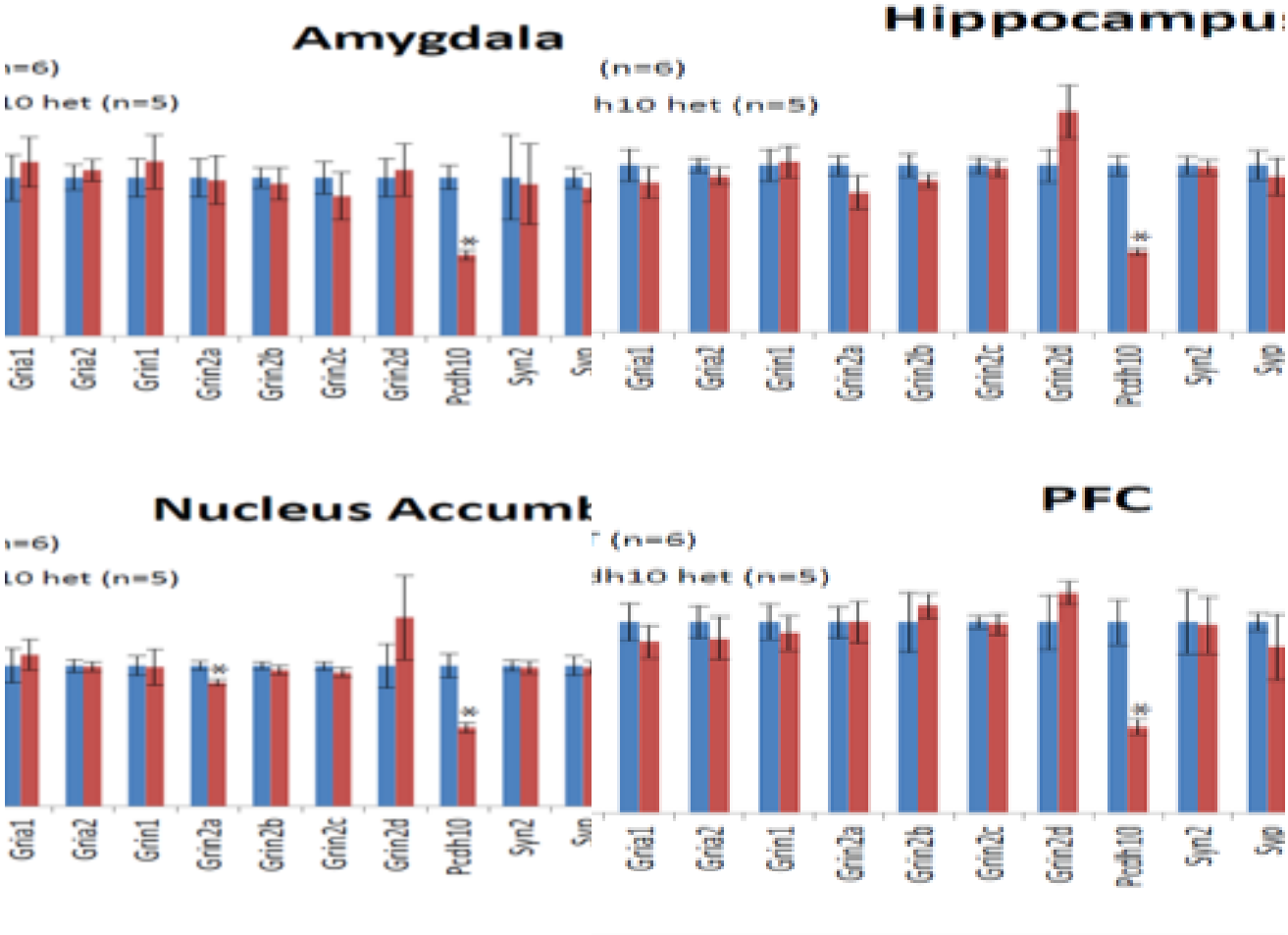

Figure 17. *Pcdh10*$^{+/-}$ mice exhibit reduced expression of Pcdh10 mRNA but no significant differences relative to WT littermates in expression of glutamatergic receptors in amygdala, nucleus accumbens, hippocampus, and prefrontal cortex (PFC). Tissue from wildtype (n=6) and Pcdh10 het (n=5) mice were RNA extracted using the Tissue Lyser system and RNeasy columns according to manufacturer's instructions (Qiagen). 1ug of RNA was converted to cDNA using the RETROscript kit (Ambion) with no heat denaturation and random decamers in the 2-step protocol. cDNA reactions were diluted to 2 ng/ul in water and RT-PCR reactions were prepared in 384-well optical reaction plates with optical adhesive covers (ABI). Each reaction was composed of 2.25ul cDNA, 2.5ul 2x Taqman Fast Universal Master Mix

(ABI), and 0.25ul of one of the following Taqman probes: Dlg4 (Mm00492193_m1), Gad2 (Mm00484623_m1), Gria1 (Mm00433753_m1), Gria2 (Mm00442822_m1), Grin1 (Mm00433790_m1), Grin2a (Mm00433802_m1), Grin2b (Mm00433820_m1), Grin2c (Mm00439180_m1), Grin2d (Mm00433822_m1), Pcdh10 (Mm00477987_s1), Syn2 (Mm00449780_m1), Syp (Mm00436850_m1), Vglut1 (Mm00812886_m1), Gapdh - Mm99999915_g1, Hprt - Mm01545399_m1, Tuba4a - Mm00849767_s1. Reactions were performed in triplicate on the Viia7 Real-Time PCR system (Life technologies). Relative quantification of gene expression was performed using the ΔΔCt method. The difference between each Ct and the average Ct for that gene was subtracted from the average of three housekeeper genes.

The investigators then went on to conduct synaptic electrophysiology experiments in the hippocampus in *Pcdh10*$^{+/-}$ mice and WT littermates.

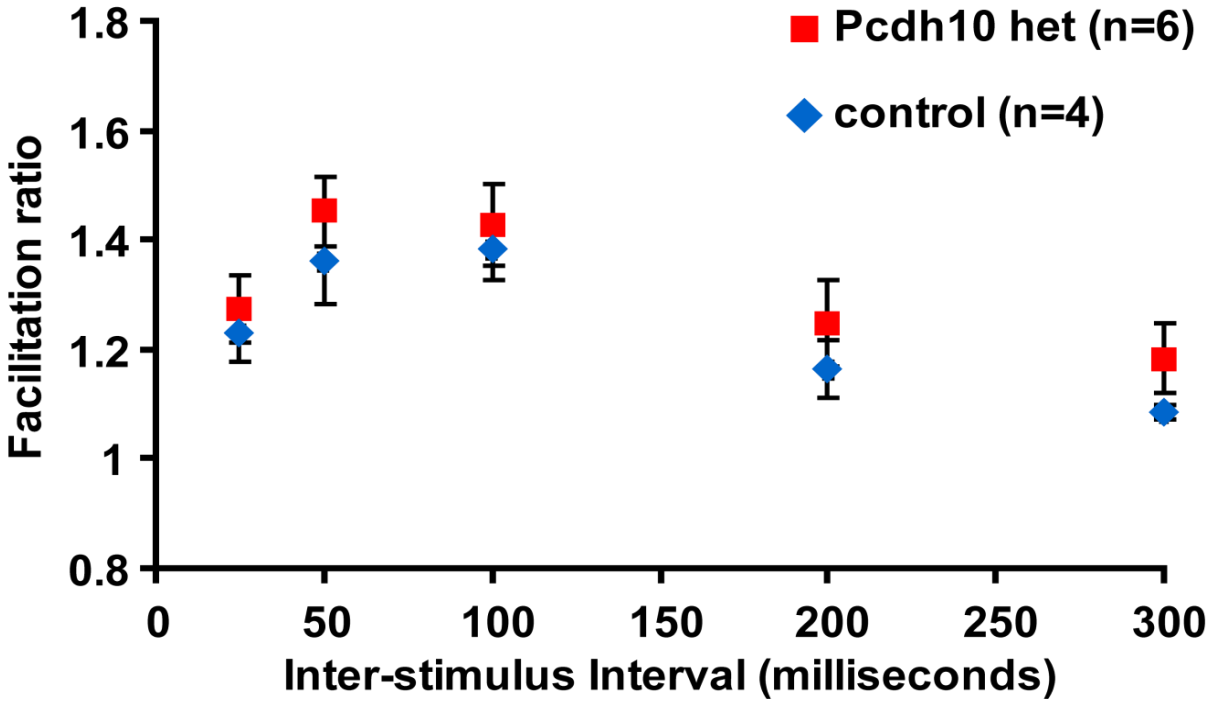

Figure 18. Paired-pulse facilitation is not changed *Pcdh10*$^{+/-}$ mice. Paired-pulse facilitation (PPF), a form of short-term plasticity and a measure of presynaptic release mechanisms, was unchanged by *Pcdh10* haploinsufficiency (p = 0.517, two-way repeated measures ANOVA, genotype and ISI as factors).

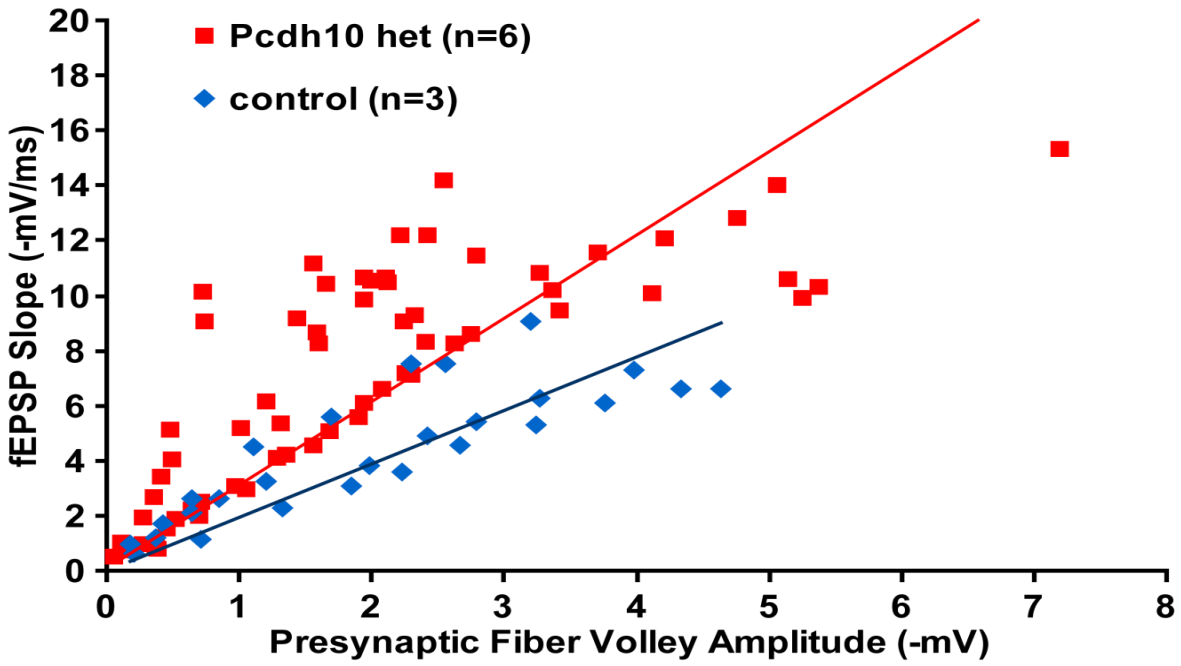

Figure 19. Input-output relationships in *Pcdh10*$^{+/-}$ mice. The slope of the field potential is plotted as a function of the pre-synaptic fiber volley. The relationship between presynaptic release and postsynaptic response is not significantly changed by *Pcdh10* haploinsufficiency (p = 0.513, t-test of average regression slopes).

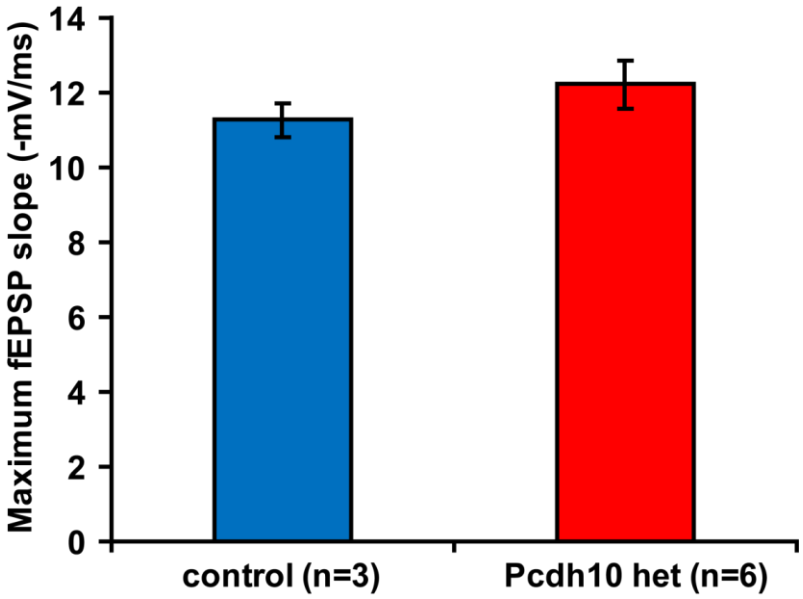

Figure 20. Maximum synaptic strength is comparable in WT and *Pcdh10*$^{+/-}$ mice. The maximum fEPSP slope elicited by stimulation was used as a measure of strength. No difference was found between genotypes (p = 0.326, t-test).

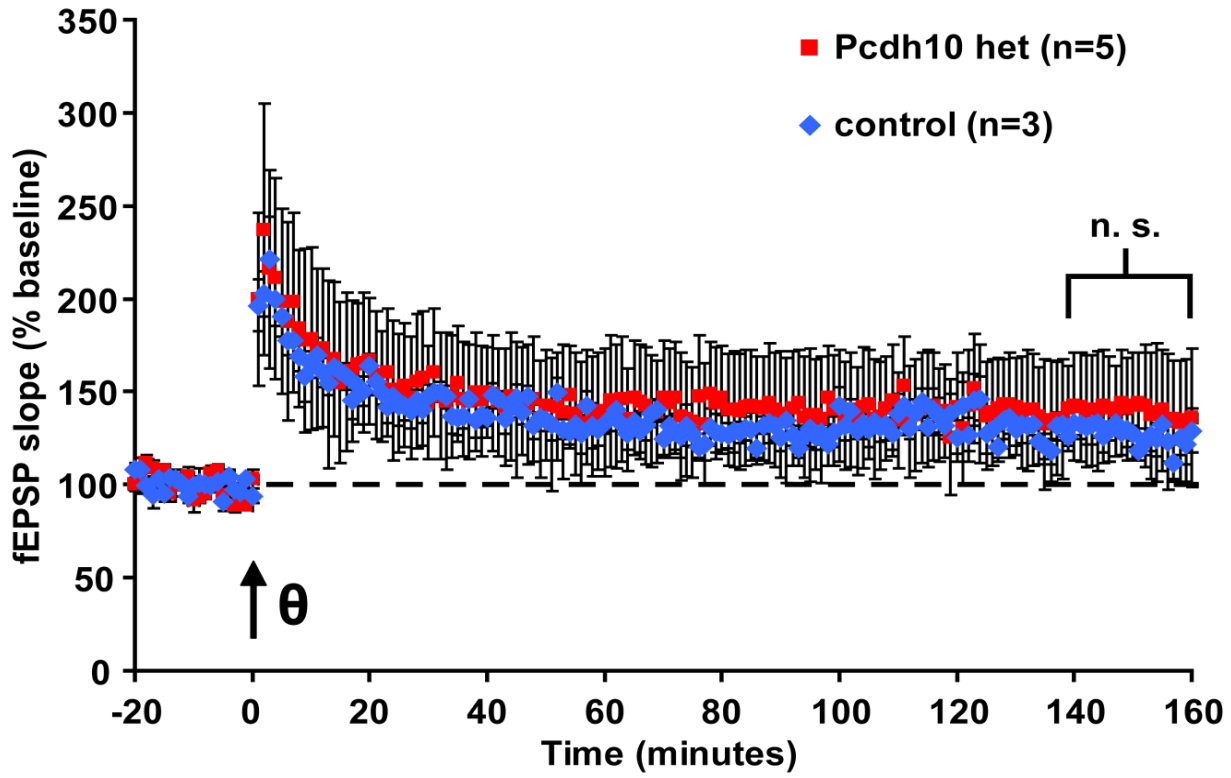

Figure 21. Theta-burst L-LTP is not changed in *Pcdh10*$^{+/-}$ mice. LTP maintenance was not significantly difference between genotypes, based on comparison of the final 20-minute epoch of the recordings (p = 0.333, 1-way repeated-measures ANOVA).

For brain imaging (MRI / DTI) experiments, the following mouse brains were collected at 30-32 days of age, and the brains have been imaged: 19 *Pcdh10*$^{+/-}$ brains and 11 WT brains. In addition, the researchers collected and imaged 8 *Pcdh10*$^{+/-}$ brains and 5 WT brains at 70-80 days of age. The analysis of this imaging data is underway for total brain volume, corpus callosum volume, and DTI parameters.

In summary, the study investigators have phenotypically characterized a mouse model relevant to ASD: the *Pcdh10*$^{+/-}$ mouse model. This model has etiological validity, in that it involves deletion of a gene that has been implicated in some forms of human ASD (Morrow et al., 2008); some elements of face validity, as it shows male-specific deficits in juvenile sociability, as well as male-specific deficits in emotional (fear) learning; and possible predictive validity, as the sociability deficit was rescued pharmacologically with d-cycloserine, which has also shown some promise in addressing the social impairments of human ASD (Posey and al., 2004). Our fos immunohistochemistry data indicate that the basolateral amygdala is activated in wildtype C57BL/6J mice by behavior in the Social Choice Task. Therefore, our future plans include studies to test the hypothesis that developmental and/or functional anomalies of basolateral amygdala glutamatergic receptors mediates the social deficits of *Pcdh10*$^{+/-}$ mice, and that various pharmacologic agents that augment glutamatergic neurotransmission (such as d-cycloserine) may rescue behavioral deficits of the mouse. These studies are likely to have translational relevance. In future studies, the researchers also intend to develop translational studies of the role of

protocadherin 10 in both social deficits and learning deficits of ASD, and test the efficacy of pharmacologic agents in rescue. The study team also intends to seek funding to continue phenotypic characterization of the Cdh10 conditional knockout mouse, as well as to develop the Cdh9 conditional knockout. Some grant applications based on this work are currently pending, and additional grant applications and papers will be submitted.

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PROJECT 4: CHARACTERIZING THE PHENOTYPES OF THOSE CARRYING RARE AND COMMON RISK ALLELES.
(PI: ROBERT SCHULTZ, PhD; CO-PI, DAVID MANDELL, ScD)

Project 4 Goals:
The aim of Project 4 was to perform diagnostic characterization and "deep phenotyping" on 400 youth with ASD and 80 Typically Developing Controls (TDCs), including 160 participants (80 TDC, 80 ASD) to be studied with brain imaging measures in Project 5. The overall goal of Project 4 was to assess relationships between phenotypic dimensions (e.g., the relationship between social motivation and face perception) and to create a rich phenotypic database for analyses of brain-behavior relationships (in collaboration with Project 5), genotype-phenotype relationships (in collaboration with Project 1), and genotype-phenotype-endophenotype (brain imaging) relationships. This goal is consistent with the newest NIMH Strategic Plan, which calls for integration of genetic, neurobiological, imaging, behavioral, and clinical data in order to clarify the underlying causes of ASD.

Project 4 Methods:
This project's deep phenotyping focused on measurements of social abilities. Framed from the social motivation hypothesis perspective (Chevallier et al., 2012; see reference list), the measurements collected allow numerous facets of this theoretical approach to be tested. The social motivation hypothesis posits that social motivation drives social perception and social cognition, and that social perception provides the inputs that allow for accurate social cognition (Chevallier et al., 2012; Grelotti et al, 2002). The project therefore divided measurement of social functioning into three domains: (a) social motivation, (b) social perception and (c) social cognition.

Primary Phenotypic Dependent Variables.

a. Social motivation was assessed primarily using gaze-tracking methodology while participants viewed movies or static images of social and non-social interactions. The primary dependent variable was the relative preference for attending to social vs. non-social components of the visual display. Visual attention is taken as a proxy for social motivation (which, as described below, is thought to be governed by reward circuitry in the ventral striatum and associated cortical regions).
b. Social perception was measured with standardized face perception tests, including measures of face identity perception and facial expression perception. Both the Benton Facial Recognition Test and the "Let's Face It" Skills Battery (split half reliabilities > .75; Wolf et al., 2008) were used to measure social perceptual skills. (Assessment tool references are available in the original grant)
c. Social cognition involves higher order planning and conceptual skills, including Theory of Mind (ToM) skills. ToM skill involves being able to readily take the perspective of another person. Social cognition was measured with two tools: The Attribution of Intention Task (AIT), and the Children's Communication Checklist-2 (CCC2). The AIT is a nonverbal ToM task involving 48 comic strips depicting a short story, divided across three conditions (16 items each): ToM, physical causality with human agents, and physical causality with object agents. Comparison of performance across conditions allows for examination of a specific deficit in ToM. The CCC2 is a parental report

checklist that measures aspects of communication, with a scale measuring social pragmatic skills.

DNA. Biological samples were collected (primarily blood, but occasionally sputum) to harvest DNA and to characterize the cadherin 9-10 intergenic region risk allele identified by the researchers in a prior GWAS study of ASD (Wang et al., 2009) that formed the overarching rationale for much of this CURE Autism Center grant. Although not part of the original grant proposal, the researchers subsequently decided to characterize four other common variants that are putative ASD risk genes: COMT, MET, CNTAP2, SLC6A4, in order to assess relationships to primary dependent variables in Projects 4 and 5.

Sample Characterization Measures. The full sample is richly characterized with a large battery of measures that can be divided into several domains (complete measure description is available in the original grant application; below is a brief cataloging):

a) Diagnostic Measures: Social Communication Questionnaire (SCQ; a parent report questionnaire collected for all ASD and TDC participants); Social Responsiveness Scale (SRS, parent report collected for all ASD and TDC participants); The Autism Diagnostic Interview – Revised (ADI-R; ASD sample only); Autism Diagnostic Observation Schedule (ADOS; ASD sample only). These tools, along with prior assessment records provided by the parents were integrated using the National Institutes of Health (NIH) Collaborative Programs of Excellence in Autism (CPEA) diagnostic guidelines to arrive at a consensus diagnosis between two experienced doctoral level clinicians for each study participant.
b) General Characterization Measures (for all ASD and TDC participants): Differential Abilities Scale, 2nd edition (DAS II– Core Cognitive Battery yields scores that are equivalent to verbal and nonverbal IQ); Edinburgh Handedness Inventory; Height, weight, head circumference; Vineland Adaptive Behavior Scale, 2nd Edition: Parent/Caregiver Rating Form; Broader Autism Phenotype Questionnaire (BAPQ, each parent completed a self-report BAPQ and an informant report BAPQ for their spouse)
c) Comorbidity Measures (for all ASD and TDC participants): The Child Symptom Inventory-4 (CASI 4; parent report checklist screener for DSM-IV disorders); Screen for Child Anxiety Related Emotional Disorders – Revised (SCARED; parent and child self-report); Repetitive Behaviors Scale – Revised (RBS-R; parent-report, ASD sample only); Children's Sleep Habits Questionnaire (parent report); Behavior Rating Inventory of Executive Function (BRIEF, parent report)

Project 4 Sample:

The original grant proposed to study 400 youth with a diagnosis of ASD and another 80 TDC matched on gender and age. **The Project successfully met its recruitment goals**. The researchers brought 678 youth into the lab across the four years of funding. Of these, 419 met research diagnostic criteria for ASD (46 female, 373 male), 106 with a community diagnosis of ASD failed to meet more rigorous research diagnostic criteria for ASD, and 153 were TDCs (33 female, 120 male). The researchers oversampled TDCs to facilitate analyses comparing phenotypic measures with the larger ASD group.

While the aims of the original Project 4 research proposal focused on comparisons within the ASD group (i.e., correlations between the putative common variant risk allele between cadherin 9 and 10), the research strategy evolved across the course of grant funding to include analyses and aims that involved direct comparisons of the TDC and ASD groups. This occurred for two reasons. First, concerns emerged that the risk variant on chromosome 5 that the researchers discovered in their 2009 publication might not generalize to new samples. Second, the original research strategy placed emphasis on correlational analyses with the full sample, which would not be ready for statistical analyses until the conclusion of funding. Thus, the researchers collected additional TDC participant data in order to mine the rich and growing phenotypic database, and to study the relationships between clinical features of ASD while comparing the two diagnostic groups. This resulted in several published papers and conference presentations that are described below.

As noted in prior annual progress reports, the Project also expanded the cohort age range from the proposed range of 6 to 10 years of age to the final sample age range of 6 to 13 years. This was necessary for several reasons. First, the researchers learned that some measurement instruments would not work well with the youngest participants, especially if they were lower functioning (i.e., had below average or impaired IQ or language functioning). Participants with a low mental age (a function of younger age and lower IQ) in particular had difficulty complying with the MRI and MEG procedures in Project 5, as well as the primary dependent measurement procedures for Project 4 (eye tracking, computerized face perception testing and the social cognitive AIT measure). Possible solutions to this problem included focusing on the older end of the originally proposed sample age range (e.g. 8-10), or expanding the age range by a few years. The researchers decided that the first option would result in an age range that was so narrow that it would be exceedingly difficult to successfully recruit and study 480 participants. Moreover, the researchers recognized that interesting scientific questions about behavioral development could be addressed with a slightly broader age range. Finally, keeping to the proposed age range of 6 to 10 years of age would have required a higher functioning sample (i.e., to ensure that the participants could complete the more demanding test components), and the researchers wanted to be sure to capture a broader range of IQ in the ASD sample to better represent the true population distribution.

Phenotypic data for the final sample has now been double entered in the study database (many measures are entered at the item level, such that the database has more than 5000 columns). Data entered at time 1 and time 2 have been compared, and when discrepant, an experienced research assistant has gone back to the paper records to resolve the error and produce a final "verified" record. This was an enormous undertaking given the number of data entry points and the size of the sample. As the researchers conduct additional analyses for specific research questions, additional data cleaning may be necessary. The table below summarizes some basic characteristics of the final sample (more than 3 million data points entered twice).

	N	AGE (mean ± SD)	SRS T (mean ± SD)	DAS GCA (mean ± SD)
ASD	419	9.4 (2.2)	79.4 (11.5)	91.1 (25.1)
TDC	153	9.8 (2.1)	41.5 (6.4)	111.6 (14.7)

One of the Specific Aims of Project 4 was to characterize 100 youth who had participated in the Philadelphia Autism Instructional Methods Study (AIMS), so as to be able to test for genetic markers that might predict who profited most from the interventions provided within the Philadelphia public school system. Of the 494 children who participated in Philly AIMS, 292 parents (59.1%) consented to be contacted for future studies and were mailed a study brochure and letter describing Project 4, co-signed by Drs. Schultz and Mandell (the PI of AIMS). Twenty-five parents (8.6% response rate) responded to this first mailing and began screening procedures, while 27 letters (9.2%) were returned to our office as "undeliverable" (e.g., because the family moved and left no forwarding address). It is important to point out that many of the families in the AIMS study come from disadvantaged circumstances. Six months later, a second mailing was sent to the remaining 240 AIMS participants, using a less detailed postcard style format designed to entice a better response because it was shorter, visually more attractive and easier to read. The second mailing yielded an additional 11 (4.6% response rate) interested participants, bringing the total recruited sample to 38 children. All Project 4 study participants were promised expert clinical evaluations and detailed written reports free of charge. When the researchers designed the study, they believed that because quality services were hard to find for these inner city families, this incentive would increase participation. Thus, the researchers were surprised by the low response rate.

In addition, the Project's recruitment process proved challenging for this group of families due to parent-reported difficulties with telephone access, literacy, and problems with computer and Internet access. Moreover, the no-show rate for scheduled families was much higher for the Philly AIMS families than the other families in the study. Assessment procedures were often modified for this participant group, with much of the diagnostic and behavioral information collected through clinicians reading the paper and pencil forms to parents or engaging in a clinical interview. Of the 38 families who responded to a mailing, 33 children participated in the daylong evaluation, which consisted of confirmation of diagnosis, social motivation research experiments, and biosample collection (either blood or saliva). These children were frequently lower functioning (e.g. diminished speech and language and cognitive levels) compared to the other Project participants; thus, they had difficulties with some study assessment procedures (e.g., imaging, computer based assessments). Extending the age range beyond age 10 to the older age ceiling of 13 was not possible, because the sample had not yet aged into this range. Based on these circumstances, the study team failed to obtain the planned total sample size of 100, and made up for this shortfall in order to meet the total sample size of 400 by over-recruiting families not involved in Philly AIMS.

Project 4 Specific Aims and Preliminary Results:
The original grant application proposed two Specific Aims.

Specific Aim 4.1 To characterize the relationship between degree of social impairment and genetic status in a large sample of children with ASD.

Specific Aim 4.2. To characterize the impact of the risk SNP on treatment outcome. In a separately funded project, implementing the "STAR" treatment program for children with ASD in the Philadelphia Public school system, the researchers will evaluate the impact of carrying the common risk SNP on treatment outcome.

Because the acquisition of the full sample of 400+ cases and 80+ TDCs was not completed until the final week of the funding period, and because data double entry (with verification) was only completed immediately prior to the due date of this final report, data analyses are ongoing, and a more complete assessment of the Project's primary Aims and hypotheses will be provided in response to the Performance Review. Nevertheless, over the past 2 years, the researchers have been able to perform many analyses with smaller subsets of data (or in some cases with novel datasets that relate to core questions addressed by this project), and this has yielded several peer-reviewed journal publications and conference presentations focused on the social motivation theme of this project. These are described below.

Project 4 Completed and Published Papers:
Below the researchers summarize each study briefly by sharing the study abstract (or a summary if the published abstract was edited for this report) and select other material from the publication in order to provide a more comprehensive description of the study.

1. Chevallier, C., Kohls, G., Troiani, V., Brodkin, E.S., Schultz, R.T. (2012). The social motivation theory of autism. *Trends in Cognitive Sciences*, *16*(4), 231-239.

Project 4 is primarily focused on understanding the social deficits in ASD. The research plan called for studying the relationships between social motivation and social perceptual skills and social cognitive skills. After a study start-up period that ironed out overall recruitment and study throughput issues, the researchers decided to write a scholarly review of the Social Motivation Hypothesis of Autism, which is the primary construct studied in this Project.

Abstract. The idea that social motivation deficits play a central role in Autism Spectrum Disorders (ASD) has gained increased interest. This constitutes a shift in autism research, which has traditionally focused more intensely on cognitive impairments, such as Theory of Mind deficits or executive dysfunction, while granting comparatively less attention to motivational factors. This review delineates the concept of social motivation and capitalizes on recent findings in several research areas to provide an integrated picture of social motivation at the behavioral, biological and evolutionary levels. The researchers conclude that some forms of ASD can be construed as an extreme case of diminished social motivation and, as such, provides a powerful model to understand humans' intrinsic drive to seek acceptance and avoid rejection.

The paper describes the literature showing that diminished social interest is one of the earliest and most persistent symptoms of autism and formally develops the 'social motivation model'. This model posits that social deficits in autism are a consequence of reduced social motivation that starts early in life and has profound developmental consequences, including fewer friendships and social isolation. Although social motivation is unlikely to be fully responsible for these unwanted social outcomes, a reduced response to social reward in children with autism could effectively thwart the development of a repertoire of skills that are needed for successful social functioning. The social motivation model suggests that early impairments in the brain's reward circuitry in children with autism reduce their motivation to seek social experiences. This lessens their experience with social interactions and lessens the attention they pay to social information, setting in motion developmental processes that ultimately deprive them of adequate

social learning opportunities. This, in turn, further disrupts brain and behavioral development. In other words, when social information is not prioritized, there are profound, cascading effects on learning about — and from — the social world. This model, if accurate, also has important implications for where to place treatment emphases. If reduced social motivation precedes and drives other developmental social skill deficits, it follows that the most effective treatments will be those that target the motivational foundation as opposed to specific social skills that are largely developmental consequences. Enhancing social motivation should open up a set of powerful social learning opportunities by making relevant elements of the social environment more salient and more rewarding. The researchers are beginning to test this hypothesis in a non-CURE funded project looking at how intranasal oxytocin can increase social motivation and promote social learning.

2. Parish-Morris, J., Chevallier, C., Tonge, N., Letzen, J., Pandey, J. & Schultz, R.T. (2013). Visual attention to dynamic faces and objects is linked to face processing skills: A combined study of children with autism and controls. *Frontiers in Psychology*, *4*(185), 1-7.

Summary. Although the extant literature on face recognition skills in ASD shows clear impairments compared to TDCs at the group level, the distribution of scores within ASD is broad. In this study, the researchers took a dimensional approach and explored how differences in social attention during an eye tracking experiment correlate with face recognition skills across ASD and TDC. Social attention was assessed using infrared eye gaze tracking during passive viewing of movies of facial expressions and objects displayed together on a computer screen (Figure 4.1). Emotional discrimination and person identity perception face processing skills were assessed using the Let's Face It Skills Battery of face perception tests. The sample was composed of 110 children, including 60 youth with ASD (7 female) and 50 TDC (12 female). The sample represents a portion of the 572 participants in the CURE sample. ASD and TDC groups were matched on non-verbal cognitive ability as measured by the Differential Ability Scales – Second Edition (ASD: 111.6; TDC: 113.7), gender ratio (ASD: 53/60 male; TDC: 38/50 male) and age in years (ASD: 11.28; TDC: 11.34).

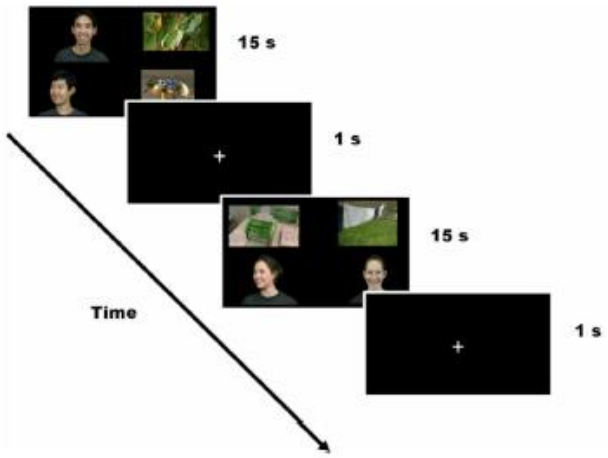

Figure 4.1. Schematic representation of eye tracking paradigm. Each "item" consisted of four movies (2 social, 2 nonsocial) playing for 15 sec each, followed by a 1 second inter-item gap with a central fixation cross hair. In a parallel study, reliability of the eye tracking procedure was assessed using 42 different children (23 with ASD, 19 TDCs, all male, mean IQ = 105) who completed the experiment twice separated by 9 weeks (±1 week). An intra-class correlation assessment of test–retest reliability for the proportion of fixations on the social stimuli (faces) was good to excellent (ICC = 0.69, $p < 0.001$).

Results: The Social Motivation Theory of autism argues that varying levels of social motivation modulate experience with faces over the course of development, and ultimately impact children's face processing skills. A two-step multiple regression analysis was used to discern whether visual attention to faces predicts face perception skill in the combined sample of ASD and TDC participants. Age was entered in Step 1, as preliminary analyses suggested that face processing skills are positively correlated with chronological age (Pearson's $r = .49$, $p < .001$). Proportion of total fixation duration to faces was entered into the model in Step 2. Consistent with our hypothesis, attention to faces accounted for a significant amount of variance in face processing skills above and beyond the effect of age, $\Delta F(1, 107) = 5.64$, $p = .02$.

Children with ASD scored significantly lower ($p < .008$) on face processing skill tests (78.83 ± 7.29) compared to matched controls (82.70 ±7.78). In addition, face processing skills in the full sample were significantly correlated with ASD symptomatology as measured by the Social Communication Questionnaire (SCQ). SCQ scores accounted for a significant amount of variance in face processing skills after accounting for the effect of age, $\Delta F(1, 107) = 23.92$, $p < .001$. However, unexpectedly, group differences in amount of attention to faces (versus objects) were not found. The researchers hypothesize that this null finding may be driven by the specific stimuli used in the eye tracking paradigm, such that the nonsocial movies were too engaging (all participants looked significantly more at objects [63%] than at faces [37%], $t(109) = -7.95$, $p < .001$), thereby making this assessment paradigm relatively less sensitive. Below, the researchers describe comparisons of sensitivity to ASD-TDC group differences for three different eye tracking paradigms used in this CURE project (McVey et al, conference abstract submitted). In summary, the researchers found that increased gaze to faces relative to objects was a significant positive predictor of face recognition skill, as would be predicted by the Social Motivation Model. There is a need for longitudinal research to truly understand how social motivation and social attention influence the development of social perceptual skills.

3. Chevallier, C., Huguet, P., Happé, F., George, N., & Conty, L. (2013). Salient social cues are prioritized in Autism Spectrum Disorders despite overall decrease in social attention. *Journal of Autism and Developmental Disorders*, *43*(7), 1642–1651.

Abstract. Diminished social attention is often considered to be a central deficit in ASDs. The researchers further investigate this hypothesis by measuring the distracting power of social and non-social stimuli in the context of a Stroop task among children with ASD and typically developing controls (TDCs). The researchers' results show that Stroop interference increases with social versus nonsocial distracters in TDCs, whereas the opposite pattern occurs in ASD. Within social stimuli, however, the superiority of direct gaze previously reported in the literature did not differ between the groups. Data thus suggest that ASD children assign less weight to social than nonsocial stimuli, but that within social signals, more salient stimuli are prioritized.

4. Chevallier, C., Parish-Morris, J., Tonge, N., Le, L., Miller, J. & Schultz R.T. (In Press). Susceptibility to the audience effect explains the performance gap between children with and without autism in a Theory of Mind task. *Journal of Experimental Psychology: General.*

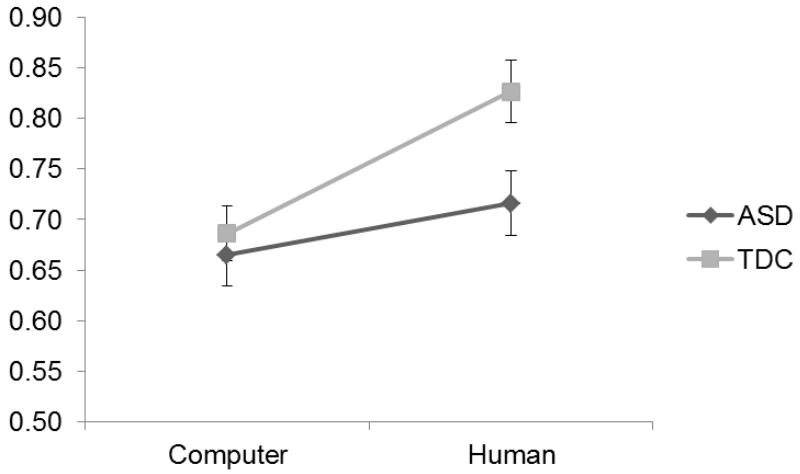

Figure 4.2. Rate of correct responses in the Computer vs. Human condition for the ASD group (dark line) compared to the TDC group (light line) in the Attribution of Intention condition.

Summary. Sensitivity to the presence of others is an important factor for successfully managing social relationships. One classic example is the 'audience effect', wherein individuals act differently when they are being watched, in a more or less conscious attempt to enhance their reputation in the eyes of others. In ASDs, diminished social motivation is thought to have a detrimental impact on these reputation management skills. In this study, the researchers hypothesized that children with ASD would be less susceptible to such audience effects, and would therefore be disadvantaged in tasks where typically developing controls (TDCs) benefit from the presence of an observer. The researchers tested this hypothesis using a Theory of Mind task (the Attribution of Attention task, AIT described above in Project Methods) administered in a social or a non-social setting (by an in person examiner vs. by computer administration, respectively). All participants were part of the CURE Autism Center Grant sample.

Results. Researchers found a diagnostic group by mode of administration interaction (see Figure 4.2), with TDCs performing better on the AIT was administered by an experimenter ("Human" condition) vs. on a computer ("Computer" condition, $t(75) = 3.40$, $p = .001$, $d=0.79$. While the TDCs benefited from testing in a social setting, children with ASD did not. This is consistent with the social motivation hypothesis because it shows a significantly diminished audience effect in the ASD group.

5. Maxwell, C.R., Parish-Morris, J., Hsin, O., Bush, J.C., & Schultz, R.T. (2013). The broad autism phenotype predicts child functioning in autism spectrum disorders. *Journal of Neurodevelopmental Disorders*, *5*(1). PMID: 24053506

Abstract: Background. Broad autism phenotype (BAP) is a milder expression of the social and communication impairments seen in ASD. However, the relationship between parental BAP traits and offspring ASD symptomatology is poorly understood. This study utilizes the Broader Autism Phenotype Questionnaire (BAPQ) in parents and the Social Responsiveness Scale (SRS) on children (parent report) to examine this connection (all study participants were from the PA

Department of Health sponsored CURE research sample). The researchers predicted that elevated maternal and paternal BAPQ scores would correlate positively with greater autism symptomatology in diagnosed children. The researchers also tested this relationship in families with typically developing children (TDC).

Method. Two hundred forty-five children with ASD, 129 TDC and all parents were recruited as part of a larger study investigating relationships between genes, brain and behavior. ADI-R, ADOS, and expert clinical judgment confirmed ASD diagnoses in children. Parents completed a self-report BAPQ and an informant report BAPQ for their spouse; an average of self and informant report for each parent was used in all analyses.

Results**:** Mothers and fathers of children with ASD had significantly higher rates of BAP traits as compared to parents of TDCs. Specifically, 21% of fathers and 10% of mothers exceeded established BAP threshold criteria in the ASD group whereas 7% of fathers and 1% of mothers did so in the TDC group ($p<0.001$ ASD vs. TDC for both maternal and paternal comparisons). Maternal and paternal BAPQ total scores were not correlated with child IQ in either group. Regression analyses showed that maternal and paternal BAPQ total scores accounted for significant variance in child SRS scores in both ASD (17.1%) and TDC (19.8%) families.

Conclusions: Our results suggest that broader autism symptomatology in parents is moderately associated with their child's autism symptomatology. This result extended to TDC families, suggesting that the BAPQ and SRS capture subtle, subclinical social variation in both children and adults. The BAPQ may be an effective tool for measuring family predisposition for ASD traits and may capture genetic background effects for ASD traits. Findings could help define multi-generational social impairments in future phenotypic and genetic studies.

Submitted Papers:

1. Troiani, V., Chevallier, C., & Schultz, R.T. Reward Associations Modulate Awareness of Novel Objects. (Submitted December 2012). *Psychological Science.*

Summary. Visual attention has a capacity limit to deal with a cluttered visual world, and thus the most salient information is often prioritized. Stimuli that are high in value attract our attention. This process is shaped by several factors, including visual experience and reinforcement history. Over time, the objects we find the most rewarding are attended more frequently. In 42 healthy

college-age adults, novel visual objects were paired with monetary reward during a training task in which one category was associated with more reward than another category (see Figure 4.3).

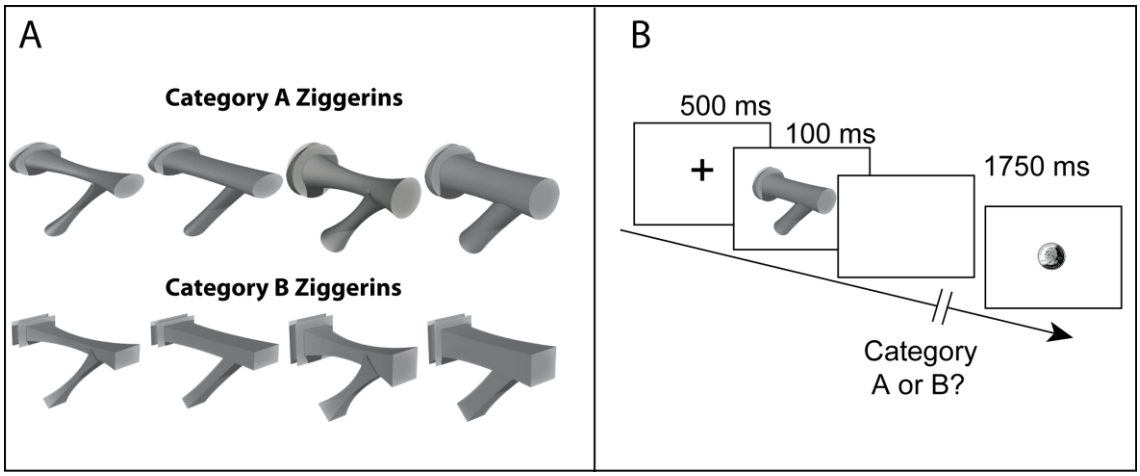

Figure 4.3. (A) Stimuli used in the current experiment. **(B)** Schematic diagram of the training task. After presentation of the object, subjects selected whether the stimulus belonged to Category A or Category B, and were differentially rewarded with money, shown by the image of a coin. This created a reward bias for either Ziggerin As or Bs.

The researchers then used a "break from continuous flash suppression" paradigm to examine whether perceptual mechanisms that control access to visual awareness incorporate the reward history of an object in prioritization of attention. Breakthrough times were significantly different between the training groups ($t(20)=-2.2$, $p=0.040$, $d=0.94$) (Figure 4.4). Thus, these results show that participants become aware of objects faster when they have been associated with a richer reward history and suggest that reward value of an object is a dimension processed prior to awareness.

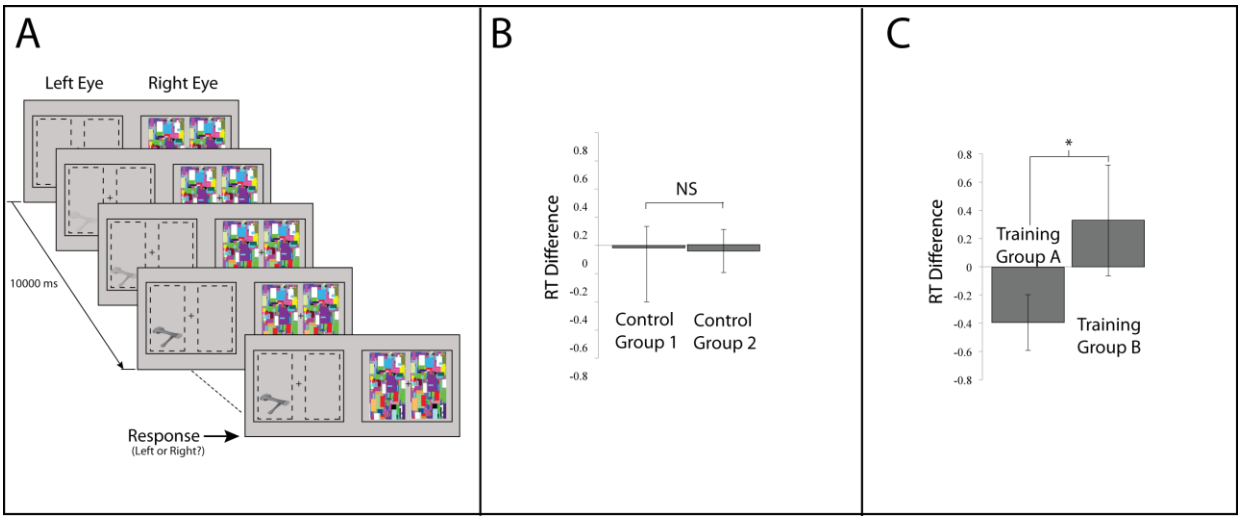

Figure 4.4. (A) Schematic representation of the continuous flash suppression test paradigm. A Ziggerin figure was gradually introduced to one eye to compete with a dynamic noise pattern presented to the other eye. The contrast of the Ziggerin figure was linearly ramped up from 0 to 100% within a period of 10 seconds from the beginning of the trial, while the contrast of the noise pattern was gradually ramped down in parallel. Observers made a response to indicate the side on which the test figure appeared. **(B)** Reaction Time difference scores for two control groups indicating no bias towards either stimulus group. This ruled out the possibility that breakthrough was based on which Ziggerin family was rewarded, because with no reward there are no differences in speed of breakthrough for these stimulus categories. **(C)** The main effect of the reward association is demonstrated in significant differences ub Reaction Time for the two training groups. Thus, when a novel object is paired with rewards during a training period, they subsequently break through to awareness more quickly to conscious awareness, i.e. they are prioritized. This has implications for why faces breakthrough faster than other objects, i.e. perhaps because a social reward history (e.g., feelings of pleasure) during social interactions.

2. Chevallier, C., Troiani, V., Schultz, R.T. Social rejection enhances preconscious processing of faces. (Submitted February 2013). *Cognition*.

Summary. Faces automatically attract attention due to their inherent social value. A wealth of research has shown that this value can be further enhanced by specific events that alter the individual's social homeostasis, e.g., by being socially excluded. However, it is not known if altered social homeostasis impacts attention to faces at the earliest, preconscious stages of visual selection. In the current study, the researchers manipulated social homeostasis in a group of 34 healthy young adults by having them recount in detail a time when they felt socially rejected or socially accepted. Participants were encouraged to relive those feelings of rejection or acceptance and to choose memories that were especially emotional.

The primary dependent measure was speed of breakthrough of visually suppressed faces (vs. a control object category – houses) using the continuous flash suppression test paradigm (see

Figures 4.4 and 4.5). House and face stimuli were matched on average luminosity (M(SD) faces = 153 (13), M(SD) houses = 158 (12), p = .19) and contrast (M(SD) faces = 48(3), M(SD) houses = 48(4), p = .16).

Results: Faces were prioritized to conscious awareness more quickly following the social exclusion prime compared to the social acceptance prime. That is, participants "saw" faces more quickly after feeling excluded, as would be predicted by the social motivation hypothesis. These results indicate enhanced unconscious processing of faces in response to personal rejection, a mechanism that potentially serves to promote interpersonal reconnection. Moreover, the researchers also measured individual differences in social anhedonia, a trait characterized by diminished pleasure derived from social interactions. Speed of face breakthrough correlated significantly with social anhedonia, r = -.56, p=0.029.

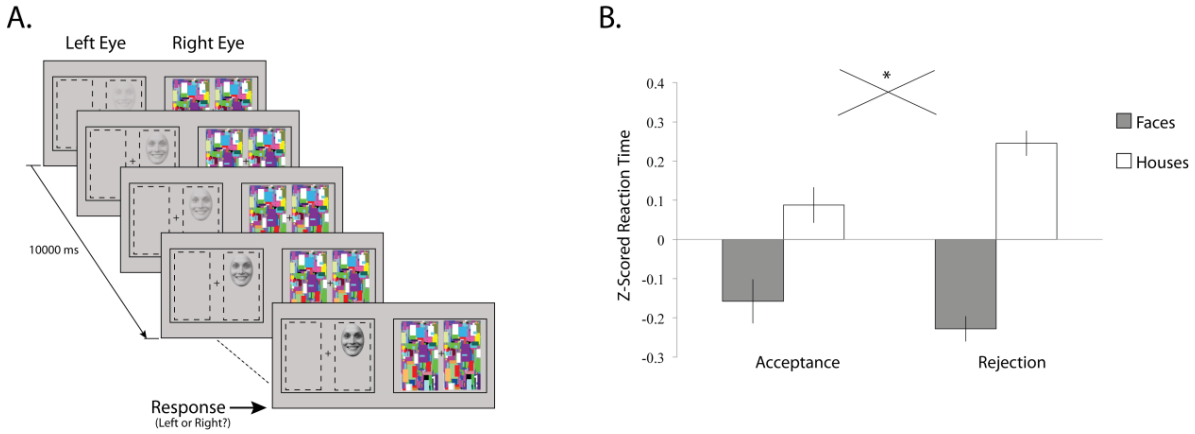

Figure 4.5. (A) Schematic representation of experimental paradigm. A test figure was gradually introduced to one eye to compete with a dynamic noise pattern presented to the other eye. The contrast of the test figure was linearly ramped up from 0 to 100% within a period of 10 seconds from the beginning of the trial, while the noise pattern was gradually ramped down in a corresponding manner. Observers made a response to indicate the side on which the test figure appeared. **(B)** Z-Scored reaction times for Acceptance and Rejection groups. Asterisk above the "x" indicates a significant condition (Acceptance or Rejection group) by stimulus type (face or house) interaction.

3. Courty, A., Maria, A-S., Lalanne, C., Ringuenet, D., Vindreau, C., Chevallier, C., Pouga, L., Pinabel, F., Philippe, A., Adrien, J-L., Barry, C., Berthoz, S. Levels of autistic traits in Anorexia Nervosa: A comparative psychometric study. (Submitted May 2013). *BMC Psychiatry*.

Abstract. Certain characteristics associated with Autism Spectrum Disorders (ASD) are over-represented among individuals with Anorexia Nervosa (AN) as well as among relatives of those with AN. Yet the co-occurrence of autistic traits in AN has still not been fully explored and no previous study has directly compared self-reported evaluations of cognitive and socio-affective skills in AN and ASD. Methods: The researchers aimed to determine the degree of overlap

between AN and ASD from scores on questionnaires classically used to measure ASD impairments. Fifteen AN subjects, 15 ASD subjects and two groups of matched controls completed a battery of self-reports measuring: autistic traits (AQ), empathy (EQ-short and IRI), systemizing (SQ-short) and alexithymia (BVAQ-B). Univariate comparisons of mean totaled scores and a Principal Component Analysis was used to study subject proximities in a reduced-factor space constructed from AQ, BVAQ and IRI subscales. Results: There appeared to be similarities in a few cognitive domains (Switching, Perspective Taking and Fantasy, lack of emotional introspection) and in some non-specific affective dimensions (depression and feelings of distress), but also marked dissimilarities in social skills (the ability to communicate emotions to others, empathizing). Conclusion: The AN and ASD subjects reported similar needs for sameness, and similar difficulties understanding their emotions and taking the perspective of another, but contrasting abilities to feel concerned in interpersonal situations. The researchers' mixed findings encourage further study of transdiagnostic similarities between these disorders.

4. Chevallier, C., Tonge, N., Troiani, V., Kohls, G., Miller, J., & Schultz, R.T. A signal detection approach to quantifying social motivation: Developing tools for the research domain criteria framework. (Submitted June 2013) *American Journal of Psychiatry*.

Abstract. Background. Recent debates in psychiatry have emphasized the need for a shift from categorical to dimensional approaches (i.e., NIHM's Research Domain Criteria [RDoc]). Of critical importance to this transformation is the availability of tools to quantify behaviors dimensionally. The present study focuses on social motivation, a dimension of behavior that is central to a range of psychiatric conditions but for which only a small number of assays currently exist.

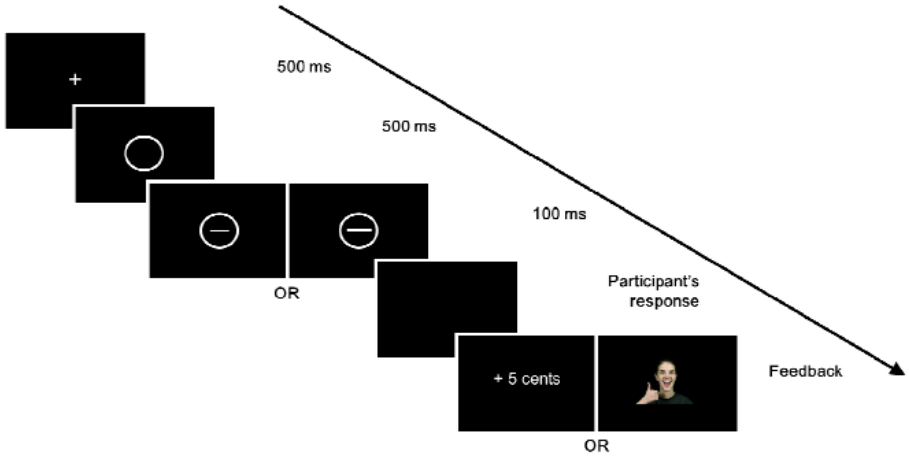

Figure 4.6. Schematic representation of the signal detection paradigm (showing a social reward). A fixation cross appears for 500ms, followed by an empty circle. A short or a long line is then flashed inside the circle for 100ms. Participants have an unlimited amount of time to respond before they receive a reward for some of their correct responses.

Method: 48 participants completed two signal detection tasks, one involving monetary rewards and the other involving social rewards. Participants also completed the Chapman Physical and Social Anhedonia Scales.

Results: Participants who scored high on the Social Anhedonia scale (indicating that they take less pleasure in social interactions) were less biased in the signal detection task by social reward (a video of a young adult actor providing social approval – a thumbs up sign, with a warm friendly smile, and an approving nod of the head). Their Social Anhedonia scores were negatively correlated with change in response bias in the social reward task but not in the monetary reward task. Conclusions: This study demonstrates that social anhedonia selectively affects formation of a response bias towards social reinforcers and constitutes an important first step towards identifying tools that can quantify social reward responsiveness dimensionally.

Notable Conference Presentations (these examples will be submitted as papers for publication this coming year):

1. Hsin, O., Souders, M., Epstein, S., Schultz, R.T. (2012, May). *The relation between poor sleep and executive functioning in children with autism spectrum disorders.* Poster presented at the International Meeting for Autism Research, Toronto, Canada.

Background: Poor sleep has been associated with executive function (EF) impairments in clinical and typical populations. Increased cognitive and behavioral rigidity, and weak working memory are aspects of EF frequently observed in ASD, as are deficits in overall sleep. Specific associations between sleep and EF among youth with ASD have not been well studied.

Objectives: Evaluate whether poor sleep is associated with executive functions in youth with and without ASD. Specifically, it was expected that: a) youth with an ASD diagnoses would be more likely to have higher ratings of EF difficulties than TDCs; b) more difficulties with sleep would be associated with worse ratings of EF; and c) the extent to which difficulties with sleep difficulties impact aspects of executive functioning would be different among youth with and without ASD.

Methods: Participants were 545 youth aged 6 to 17 with ASD (*n*= 370; 90% male; mean age 9.9±2.8) or TDC (*n*=175; 81% male; mean age =10.7 ± 3.0). Nearly all participants were part of the CURE grant sample. ASD diagnoses were made based on ADOS, ADI-R, and clinical judgment. IQ was assessed with the DAS II (ASD=92.8; TDC=112.9). Parents completed (1) the Behavior Rating Inventory of Executive Function (BRIEF) which yielded measures of inhibition (ASD=64.0; TDC=44.5), cognitive and behavioral rigidity (Shift Scale; ASD=69.1 TDC=43.5), emotional control/lability (ASD=62.1; TDC=43.7), ability to initiate or start tasks (ASD=63.7; TDC=44.4), working memory (ASD=65.3; TDC=45.9), planning (ASD=63.3; TDC=44.1), organization (ASD=57.8; TDC=47.2), self-monitoring (ASD=65.1; 42.7), and overall executive function impairment (Global Executive Composite; ASD=67.1; TDC=43.1) (higher scores indicated greater impairment); (2) the Children's Sleep Habits Questionnaire (CSHQ) which yielded a total subscale score (ASD=44.8; TDC=40.2; higher score indicated poorer sleep).

Results: Youth with ASD had significantly more difficulties with sleep and executive functions than the TDC group. Main effects were found, such that group (ASD) and sleep were both

significant independent "predictors" for all aspects of executive functioning ($p<.001$ for all β's). Poorer sleep accounted for 2.3-3.6% of the variance in aspects of EF. Significant moderations for group were found for the Initiate subscale of the BRIEF.

Conclusions: Poorer sleep was associated with more impaired Inhibition, Shifting, Emotion Control, Initiation, Working Memory, Planning, Organizing, and Monitoring. These difficulties were above and beyond what could be explained by having an ASD alone. These scores reflect functions that are associated with the core deficits in ASD that have a significant impact on academic, community, and social functioning. The researchers hypothesize that poor sleep causes problems with day-to-day cognitive and emotional control that negatively impacts adaptive functioning and quality of life, though this could not be directly tested in this cross sectional, correlational study design. If true, more effort should be devoted to sleep intervention for ASD.

2. Granader, Y., Yerys, B. E., Wallace, G. L., Lawson, R., Rosenthal, M., Wills, M., Dixon, E., Anthony, L.G., Pandey, J., Thompson, R., Schultz, R. T. & Kenworthy, L. *The Factor Structure of the Behavior Rating Inventory of Executive Function in Children and Adolescents with Autism Spectrum Disorders Replicates the Normative Sample*. Poster presented at the International Meeting for Autism Research, San Sebastian, Spain, May 2013

Background: Children with autism spectrum disorders (ASDs) have a high rate of executive functioning (EF) deficits, which can cause great difficulty in daily activities at both school and home. Given that EF weaknesses are more common in children with ASDs than in the general population, it is important to screen for executive dysfunction in order to recommend appropriate clinical interventions. The Behavior Rating Inventory of Executive Function (BRIEF) assesses EF skills in daily life. The psychometric properties and clinical utility of the BRIEF have been examined in clinical populations. Elevated BRIEF scores have been reported in ASD; however, the factor structure of the BRIEF has not been explored in this population.

Objectives: To investigate the factor structure of the BRIEF in a large sample of children with ASDs and to determine the frequency of BRIEF scale elevations in this sample.

Methods: 479 children with ASDs (405 males and 74 females, age range 5-18 years, mean = 10.6, SD = 3.2) were assessed at Children's National Medical Center, National Institute of Mental Health, and the Children's Hospital of Philadelphia. CHOP participants were all part of the CURE grant cohort. Participants with an IQ below 70 were excluded from this study. IQ ranged from 70-158 (mean = 101.0, SD = 17.7). Parents completed the BRIEF. BRIEF scale elevations were determined to be of "potential clinical significance" if the T-score was at or above 65. Principal component analyses (PCA) with oblique (promax) rotations were performed on the BRIEF subscale T-scores in order to explore the underlying structure.

Results: Subscale elevations were generally consistent with previous research in ASD. The Shift, Initiate, Working Memory, Plan/Organize, and Monitor subscales were elevated in 51 to 63% of the sample. The PCA revealed one factor that accounted for 57.1% of the variance. When a two-factor solution was forced factor loadings ranged from .54 to .97. Five of the scales loaded highly on the first component (Plan/Organize, Working Memory, Organization of

Materials, Initiate, and Monitor) and three of the scales loaded predominantly on the second component (Emotional Control, Shift, and Inhibit). No cross factor loadings were found. The two constructs defined by these component loadings replicated the original normative data in the BRIEF manual identifying two indices: Behavioral Regulation and Metacognition.

Conclusions: This study provides psychometric and clinical descriptive data about the BRIEF subscale scores in a large sample of children with ASDs. The results of this study are similar to previous findings, which indicate that a substantial proportion of children with ASDs display significant difficulties with EF as measured by the BRIEF subscales. In addition, the PCA revealed one factor that corresponded to the original Global Executive Composite and when a two-factor solution was forced the factor structure corresponded to the original Behavioral Regulation and Metacognition Indices, and provided validation for using normative BRIEF data in studies of children with ASDs. Future studies should use confirmatory factor analysis in order to further examine the validity of the BRIEF with this population.

3. McVey, A., Schultz, R., Parish-Morris, J., Pandey, J., Chevallier, C. (Submitted). *Studying social attention in ASD: Stimulus type matters.* Poster to be presented at the annual meeting of Object Perception, Attention, and Memory (OPAM), Nov. 14, 2013, Toronto, ON, CA

In published paper #2 (Parrish-Morris et al, 2013) described above, the researchers note that the eye tracking procedure used in that study of CURE participants predicted face processing skills on an independent test, but surprisingly failed to show ASD vs. TDC group differences. In this presentation, the researchers now explore eye tracking methodological factors that make these paradigms more or less sensitive to putative group differences.

Abstract**:** ASD is characterized by social impairments, which have been related to deficits in social attention. People with ASD have indeed been found to attend to faces less than control participants. Yet, eye-tracking studies vary in how well they uncover these social attention deficits. In this study, children with and without ASD from the PA CURE sample participated in three eye-tracking tasks that differed on the ecological relevance of the social stimuli (i.e., static vs. dynamic vs. interacting faces). The researchers found that interacting faces were best at differentiating ASD from controls, whereas tasks including dynamic and static stimuli had lower sensitivity and specificity.

Background: An extensive body of literature suggests that people with ASD attend to faces less than TDCs, and concomitantly, that they have unusually high interest in certain categories of non-social objects (trains, for instance). Eye-tracking is a common method for examining social perception and preferences in ASD, but eye-tracking studies vary in how well they uncover social attention deficits. Riby and colleagues, for instance, used dynamic cartoon and human actor videos and found that children with ASD attend less to the faces and more to the non-social background area than TD children (Riby & Hancock, 2009). On the other hand, Van der Geest and colleagues conducted several studies utilizing static images of cartoon characters and human actors, and found no significant difference between the social responses of children with ASD compared to TD controls (Van der Geest et al, 2002a,b). Such inconsistencies between studies may be due to the varying methods, designs, and stimulus types employed (Saitovitch et al., 2013). In particular, static stimuli may not be as potent as dynamic stimuli in measuring

individual differences in social attention. Similarly, scenes depicting more or less ecological social interactions will likely vary in their ability to elicit social responses. The researchers hypothesize that the nature of the social stimuli will have an impact on participants' social response and that highly ecological, dynamic stimuli will be optimal to uncover meaningful differences in social attention between ASD and TD controls.

In order to explore this question, the researchers capitalized on existing data collected as part the Penn/CHOP CURE cohort of participants who took part in one or more of three eye-tracking experiments using several types of stimuli. These stimuli included static vs. dynamic stimuli and more vs. less ecological interactive settings. This large dataset allowed the researchers to aggregate a subset of participants who had taken part in all three experiments so as to directly investigate the impact of stimulus type on a task's ability to identify group differences in social attention.

Methods: 72 children with an ASD (mean age = 11.9 years) and 34 TD children (mean age = 14.7 years) participated in a set of three eye-tracking paradigms. Eye gaze was tracked with a Tobii-T120 eye-tracker. The study consisted of a "Static Visual Exploration" task in which static images of objects and people were presented (the original paradigm taken from Sasson et al, 2008 and described in the original CURE grant application); a "Dynamic Visual Exploration" task in which four dynamic video clips of individual faces and objects were simultaneously presented (see Parish-Morris et al 2013, described above under published papers); and an "Interactive Visual Exploration" task in which highly ecological video clips of children playing together were presented (created for use in Project 5 of the CURE grant during fMRI, and subsequently used in later years in Project 4 outside of the MRI).

Results: The researchers found that each of these three eye-tracking paradigms did not differentiate ASD from TDC with equal efficacy. Repeated measures ANOVAs controlling for age revealed that children with ASD spent less time looking at faces than TD controls in the Interactive Visual Exploration task, but not in the other two tasks. ROC Curves using total fixation duration to faces to examine the sensitivity and specificity of each task in distinguishing the ASD group from the TD group showed that the Interactive Visual Exploration was superior; it had the highest sensitivity and specificity. This paradigm contained dynamic videos of school aged children playing. Visual Exploration had poor sensitivity and specificity, and was the weakest at measuring social response.

Conclusions: The use of eye-tracking paradigms to evaluate various gaze behaviors is growing across fields of study. It is important to note, therefore, that not all eye-tracking paradigms are of equal efficacy in measuring ASD. Dynamic stimuli appear to be better than static images for measuring social response, and, in particular highly ecological paradigms presenting actual interactive scenes were found to be optimal stimuli.

Project 4 Conference Presentations

1. Pandey, J., Kang, H. W., Giserman, I., Bradstreet, L., Cayless, S. J., and Schultz, R.T. (May 2011). The assessment of adaptive functioning in children and adolescents with ASD: A comparison of two widely used measures. Presented at International Society for Autism

Research, San Diego California.

2. Puleo, C., Schultz, R.T., and Kendell, P.C. Characteristics of anxiety disordered children with symptoms of autism. (May 2011). Poster Presented at International Society for Autism Research, San Diego California.
3. Taylor, J.M., Schultz, R.T., Riley, M., Hunyadi, E.T., J. Letzen and Herrington, J.D. (May 2011). Individuals with ASD and co-occurring anxiety show increased amygdala and orbitofrontal cortex activity during face perception. Presented at the International Meeting for Autism Research, San Diego California.
4. Le, L., Parish-Morris, J., Sasson, N., and Schultz, R.T. (May 2012). Theory of mind in children with autism spectrum disorder: a "non-verbal" task highlights the importance of language. Presented at the International Meeting for Autism Research, Toronto, Canada.
5. McDermott, M.H., Kang, H.W., Parish-Morris, J., Chevallier, C., Bush, J.C., and Schultz, R.T. (May 2012) Eye-tracking established as a reliable test-retest measure in adolescents with ASD: visual attention to social and non-social stimuli. Presented at the International Meeting for Autism Research, Toronto, Canada.
6. Mosner, M.G., Bradstreet, L. E., Guy, L., Schaaf, R. C., Schultz, R.T. and Souders, M. (May 2012). Correlations between sensory processing symptoms and sleep disturbances among children with autism spectrum disorders. Presented at the International Meeting for Autism Research, Toronto, Canada.
7. Hsin, O., Souders, M., Schultz, R., and Epstein, S. (May 2012). The relation between poor sleep and executive functioning in children with autism spectrum disorders. Presented at the International Meeting for Autism Research, Toronto, Canada.
8. Bush, J. C., Chevallier, C., Rump, K., Parish-Morris, J. and Schultz, R. T. (May 2012). Face processing and its correlation to theory of mind in autism spectrum disorders. Presented at the International Meeting for Autism Research, Toronto, Canada.
9. Tonge, N. A., Chevallier, C., Parish-Morris, J., Letzen, J. and Schultz, R.T. (May 2012). Social motivation is correlated with face processing skill in children with ASD. Presented at the International Meeting for Autism Research, Toronto, Canada.
10. Souders, M. C., Puleo, C. M., Bennett, A., Berry, L. N., Giserman, I., Eriksen, W. T., Schultz, R.T. and Herrington, J.D. (May 2013). Potential link between anxiety and insomnia in individuals with autism spectrum disorders. Presented at the International Meeting for Autism Research, San Sebastian, Spain.
11. Bush, J.C., Maxwell, C.R., Hsin O. and Schultz, R.T. (May 2013). BAPQ as a predictor of child functioning in autism spectrum disorders. Presented at the International Meeting for Autism Research, San Sebastian, Spain.
12. Tonge, N., Schultz, R.T., Troiani, V., Kohls, G., Miller, J. and Chevallier, C. (May 2013). A signal detection approach to quantifying social motivation in adults. Presented at the annual meeting of the American Psychological Science Society, Washington DC.
13. Bush, J., Schultz, R.T., Paterson, S., Parish-Morris, J., Surian, L., Chevallier, C. (May 2013). Measuring theory of mind in low functioning children with autism using anticipatory looking. Presented at the annual meeting of the American Psychological Science Society, Washington DC.

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PROJECT V: CHARACTERIZING THE BRAIN ENDOPHENOTYPES OF THOSE CARRYING RARE AND COMMON RISK ALLELES.
(PI: ROBERT SCHULTZ; CO-PIs: PhD RAGINI VERMA, PhD, TIMOTHY ROBERTS, PhD)

Project 5 Overview of Goals and Progress:
The goal of Project 5 as outlined in the original grant application was to study with MRI and magnetoencephalography (MEG) 80 youth with ASD and 80 matched typically developing controls (TDCs) characterized in Projects 1 and 4. Project 4 provided the subject recruitment, diagnostic characterization and deep phenotyping for all of the other Projects. Project 1 studied the ASD common variant discovered by Wang et al (2009) in the inter-genic region between cadherin 9 and 10 on chromosome 5 and passed that genotypic information on that variant to Projects 4 and 5. Project 5 focused on studying group differences in brain structure, using structural MRI (sMRI) and diffusion tensor imaging (DTI), and brain function using fMRI and MEG. The focus of the analyses has been on differences in brain structure and function during social processing tasks, primarily face perception tasks, as well as during resting states.

The original aims called for performing neuroimaging on 80 youth with ASD and 80 who are TDCs between ages of 6 and 10 years. However, younger children in this age range frequently moved during scanning, producing data that often was not useable. Thus, as noted in a prior annual progress report, the researchers expanded the recruitment age up to 13 years (see also the

Progress Report for Project 4 for additional information on the rationale for this change from the perspective of phenotypic data collection).

Recruitment for Project 5 was delayed by MRI and MEG equipment orders that failed to arrive on time (see Sample description below). Overall, recruitment was more difficult and slower than anticipated; nevertheless **the researchers were able to meet their MRI scanning goals, and successfully studied a total of 164 participants (81 with ASD and 83 with TDC)**. MEG recruitment and scanning fell slightly short (n=150 with 79 ASD and 71 TDC) since new equipment was even further delayed in its arrival and installation compared to MRI. All participants who completed the MEG study also completed the MRI study. The researchers have been able to add additional TDC and ASD MRI participants from several parallel MRI studies at the Center for Autism Research (CAR); when possible the identical face processing fMRI tasks as used in Project 5 were added to these other protocols to provide a much larger sample for the final analyses.

Data collection continued until late May, 2013, the end of grant funding. Image processing and preliminary statistical analyses are ongoing, but are not yet complete. The researchers expect to have a more complete description of the Project results by the time of the Performance Review. Phenotypic data for the final sample for Projects 4 and 5 have now been double-entered in the study database (many measures are entered at the item level, such that the database has more than 5000 columns). Data was double-entered by independent personnel, and those two data sets were compared. When discrepant, a senior research assistant went back to the paper records to resolve the error and produce a final "verified" record. This was an enormous undertaking given the number of data entry points and the size of the sample. As the researchers conduct additional analyses with the phenotypic database for specific research questions, additional data cleaning may become necessary.

In this report the researchers describe analyses accomplished thus far, and on interim analyses that resulted in published papers or conference presentations using portions of the final sample. In some cases other data was employed in the researchers' analyses. These are described in this report because they were conducted by Project personnel and have direct bearing on planned analyses for this Project. For example, the researchers report below on method advancements with portions of the total CURE sample or with independent samples, in service of methods to be used this CURE Center Grant. The progress report largely focuses on analyses that resulted in published papers or conference presentations. These methodological studies are of great importance and allow the researchers to employ the most advanced techniques to the analyses of the full and final CURE imaging data set.

Project 5 Sample:
The original grant reported that MRI data would be collected on a new research-dedicated Siemens Verio 3T. This order was delayed, as described in an earlier progress report, which delayed data collection. Nevertheless, the researchers were able to catch up and meet the recruitment goals. The MEG studies, however, were more seriously delayed by a new equipment order. As described in prior progress reports, MEG scanning did not start at the same time as MRI scanning, but rather was lagged by about 6 months. Efforts were made to bring all participants back who had completed MRI to do MEG, but this was only partially successfully.

The final MEG sample fell short of the goal of 160 participants. Instead, MEG data were collected on a total of 150 participants, including 71 TDCs and 79 with ASD.

All study participants came through Project 4 and were fully characterized with gold standard diagnostic procedures (ADOS, ADI-R) following CPEA guidelines, and had measurements of full scale IQ, adaptive behavior, psychiatric comorbidities, executive functioning, sleep, communication abilities, as well as a more detailed experimental battery of measures focused on social abilities. The latter included measures of:

(a) Social motivation (assessed primarily using gaze-tracking methodology while participants viewed movies or static images of social and non-social interactions). The primary dependent variable is the relative preference for attending to social vs. non-social components of the visual display. Visual attention is taken as a proxy for social motivation.
(b) Social perception was measured with standardized face perception tests, including measures face identity perception and facial expression perception. Both the Benton Test of Facial Recognition and the Let's Face Skills Battery (split half reliabilities > .75; Wolf et al., 2008) were used to measure social perceptual skills. (Assessment tool references are available in the original grant)
(c) Social cognition involves higher order planning and conceptual skills, including Theory of Mind (ToM) skills. ToM skill involves being able to readily take the perspective of another person. Social cognition was measured with two tools: The Attribution of Intention Task, and the Children's Communication Checklist-2 (CCC2).

The Final Progress Report for Project 4 and the original grant application contains additional information about the phenotypic assessments.

DNA. Biological samples were collected (primarily blood, but occasionally sputum) to harvest DNA and to characterize the cadherin 9-10 intergenic region risk allele identified by the researchers in a prior GWAS study of ASD (Wang et al. 2009) that formed the overarching rationale for much of this CURE Center grant. Although not part of the original grant proposal, the researchers subsequently decided to characterize four other common variants that are putative ASD risk genes: COMT, MET, CNTAP2, SLC6A4, in order to assess relationships to primary dependent variables in Projects 4 and 5. Some but not all of this extra genotyping is complete; the remainder is expected to be completed by September, 2013.

Project 5 Specific Aims and Preliminary Results:

The original grant application proposed three Specific Aims. Aims 1 and 2 concerned unimodal data analyses, while Aim 3 concerned multimodal pattern classification. Analyses of the CURE data for Aim 3 won't begin until after unimodal data analyses are nearly complete, which is expected in the last quarter of 2013.

Specific Aim 5.1 Progress:

Specific Aim 5.1: To use structural MRI and diffusion tensor imaging to characterize aberrations in brain structure among children with an ASD, and examine how these might be related to genetic risk factors.

Methods. MRI data for Project 5 was collected on CHOP's research dedicated 3T Verio scanner. Structural MP RAGE scans were collected with 0.9 X 0.8 X 0.8 mm voxel resolution, and with TR/TE=2000/3.3ms. Structural data were first processed using Davatzikos and colleagues' RAVENS (Davatzikos et al., 2001) method for deformation based assessment of structural differences between groups. One advantage of RAVENS with respect to comparable techniques (such as SPM's voxel-based morphometry) is that it preserves the amount of GM, WM and CSF present in an individual's scan, thereby allowing for robust local volumetric analysis. DTI data were collected using a 30-direction protocol per the original grant application (2 mm isotropic voxel size, TR/TE=13900/68 ms, Matrix=130x130). However, early on in data collection, the researcher's leveraged CURE funding to earn an NIH R01 grant award to study High Angular Resolution Diffusion Imaging (HARDI) data in ASD and TDC (PI: Verma). Whenever time permitted, HARDI acquisition was added to the CURE participant data acquisition. DTI data are being processed using the pipeline developed by Dr. Verma, as described in the original grant application and below in presentation of results obtained thus far.

While waiting for the full sample to be ready for analyses, researchers have been productive in using available data across the past few years for both clinical and methodological papers. This section provides publication citations, abstracts, and other information to complete the description of the research methods and findings. With respect to Aim 5.1, researchers have published:

- two papers using portions of the final sMRI data set,
- two review papers describing ASD brain imaging research
- six DTI papers focused on method development (methods which are being used in final DTI analyses), and
- one paper that is currently under a second round of review at *Neuroimage* about a new method for characterizing ASD heterogeneity across a single severity metric that is noteworthy for its utility in improving group separation in imaging studies.

Each paper that did not mistakenly neglect to cite funding from PA Department of Health is included in the publication appendix that accompanies the text of this report. Below we summarize these papers.

1. Varol, E., Gaonkar, B., Erus, G., Schultz, R., Davatzikos, C. (2012). Feature ranking based nested support vector machine ensemble for medical image classification *Proceedings - International Symposium on Biomedical Imaging (ISBI)*, 146-149.

Abstract: This paper presents a method for classification of structural magnetic resonance images (MRI) of the brain. An ensemble of linear support vector machine classifiers (SVMs) is used for classifying a subject as either patient or healthy control. Image voxels are first ranked based on the voxel wise t-statistics between the voxel intensity values and class labels. Then voxel subsets are selected based on the rank value using a forward feature selection scheme. Finally, an SVM classifier is trained on each subset of image voxels. The class label of a test subject is calculated by combining individual decisions of the SVM classifiers using a voting mechanism. The method is applied for classifying patients with neurological diseases such as Alzheimer's disease (AD) and autism spectrum disorder (ASD). The results on both datasets

demonstrate superior performance as compared to two state of the art methods for medical image classification.

Additional information from the publication: One major challenge of medical image classification is the very high dimensionality of the image domain. A typical MRI scan of the brain includes several million measurements (image voxels). In order to obtain optimal performance on multiple datasets, a classifier should be trained on features that capture different patterns of structural deviations, going from very localized to completely global. With this aim, researchers extend and improve upon the supervised classification framework by using two established concepts of machine learning, a) ensemble learning (done by training multiple classifiers on different feature sets), and b) feature ranking. In this work, researchers use a feature ranking strategy as a first step for constructing nested feature subsets. The features with the highest ranking are grouped in the first feature subset. Each subsequent subset extends the previous one by adding less discriminative features, until all features are included. Each support vector classifier in the ensemble is trained on one of these feature vectors.

The method was been applied to two different neurological disorders: Alzheimer's disease (AD) and ASD. The ASD data included scans of a total of 131 different male subjects (81 ASD / 50 TDC). The images were T1 MR scans. The mean age of ASD subjects were 11.8 and standard deviation was 3.0. The results of both datasets demonstrated superior performance as compared to two state of the art methods (Kloppel's and COMPARE) for medical image classification. See Figure 5.1 from the paper, presented below. This study showed that aggregating classifiers trained on nested feature sets attained better classification performance than classifiers trained on a single feature set.

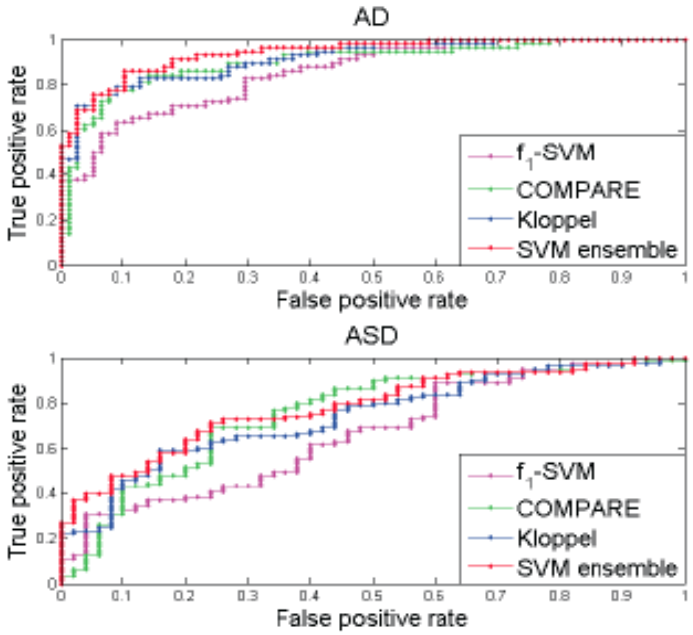


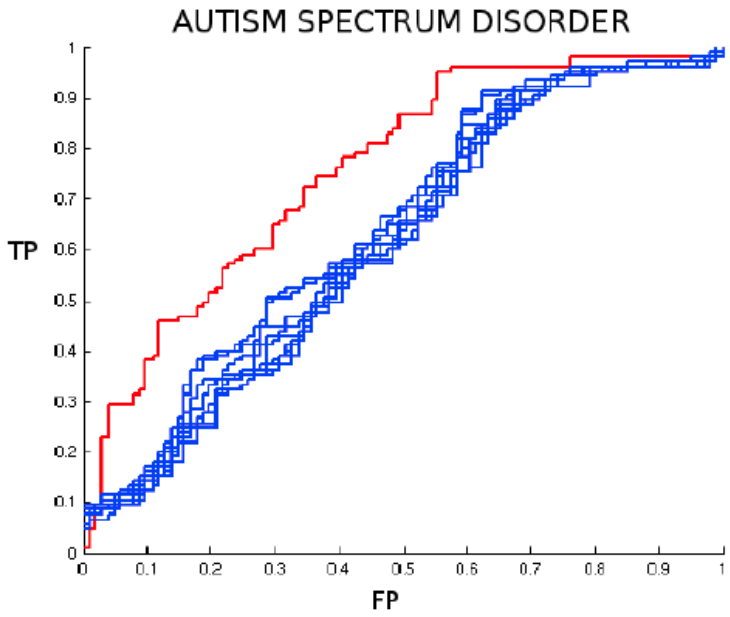
Figure 5.1. ROC curves for the classification by different methods, showing that the researchers' method (aggregating classifiers trained on nested feature) outperforms others in the literature.

2. Varol, E., Gaonkar, B, Davatzikos, C. (2013). Classifying medical images using morphological appearance manifolds. *Proceedings - International Symposium on Biomedical Imaging (ISBI)*, 740-743.

Abstract. Input features for medical image classification algorithms are extracted from raw images using a series of pre-processing steps. One common preprocessing step in computational neuroanatomy and functional brain mapping is the nonlinear registration of raw images to a common template space. Typically, the registration methods used are parametric and their output varies greatly with changes in parameters. Most results reported previously perform registration using a fixed parameter setting and use the results as input to the subsequent classification step. The variation in registration results due to choice of parameters thus translates to variation of performance of the classifiers that depend on the registration step for input. Analogous issues have been investigated in the computer vision literature, where image appearance varies with pose and illumination, thereby making classification vulnerable to these confounding parameters. The proposed methodology addresses this issue by sampling image appearances as registration parameters vary, and shows that better classification accuracies can be obtained this way, compared to the conventional approach.

Additional Summary Information. The problem of classifying an individual into one of two or more classes (e.g. healthy vs. ASD) using a medical scan is important for understand the neurobiology of ASD and for early detection and monitoring change over time (and perhaps across successful treatment). Attempting to find parameters to maximize classifier performance is an extremely difficult and computationally intensive task. This paper describes methodology adapted from the computer vision and medical imaging literatures that has shown that understanding the image appearance manifold formed by sampling the parameter space (i.e., the parameters affecting registration accuracy) improves the richness of the representation of each object, and can therefore increase our ability to recognize an individual in a way that is robust relative to these parameters. The ASD dataset (105 patients / 101 controls) were taken from the CURE Autism Center grant cohort and related grants at the Center for Autism Research, and consisted of T1-weighted MR scans from a 3T scanner, acquired sagitally using volumetric 3D MPRAGE with 0.8 mm X 0.8 mm in plane resolution and 0.9 mm thick sagittal slices. To circumvent the problem of finding the optimal registration, the researchers build upon the Anatomical Equivalence Class (AEC) concept, sampled multiple morphological appearances (CMD's) of subjects from AECs as a means of enriching training sets for improved classification. Figure 5.2 shows the resulting receiver operator curve (ROC), demonstrating improved classification of ASD using this approach. This new approach that can be exploited to improve medical imaging based pattern classification.

Figure 5.2. ROC curve for ASD classification. Blue indicates for classifiers trained with one CMD per individual for varying. Red indicate the classifier performance when the training and testing sets are extended to include all CMD's per individual, and testing classification aggregated through unweighted voting.



3. Kohls, G., Chevallier, C., Troiani, V., & Schultz, R.T. (2012). Social 'wanting' dysfunction in autism: Neurobiological underpinnings and treatment implications. *Journal of Neurodevelopmental Disorders, 4*(7), 1-20.

Summary. Most behavioral training regimens in autism spectrum disorders (ASD) rely on reward-based reinforcement strategies. Although proven to significantly increase both cognitive and social outcomes and successfully reduce aberrant behaviors, this approach fails to benefit a substantial number of affected individuals. Given the enormous amount of clinical and financial resources devoted to behavioral interventions, there is a surprisingly large gap in our knowledge of the basic reward mechanisms of learning in ASD. Understanding the mechanisms for reward responsiveness and reinforcement-based learning is urgently needed to better inform modifications that might improve current treatments. The fundamental goal of this review is to present a fine-grained literature analysis of reward function in ASD with reference to a validated

neurobiological model of reward: the 'wanting'/'liking' framework. Despite some inconsistencies within the available literature, the evaluation across three converging sets of neurobiological data (neuroimaging, electrophysiological recordings, and neurochemical measures) reveals good evidence for disrupted reward-seeking tendencies in ASD, particularly in social contexts. This is most likely caused by dysfunction of the dopaminergic–oxytocinergic 'wanting' circuitry, including the ventral striatum, amygdala, and ventromedial prefrontal cortex. Such a conclusion is consistent with predictions derived from diagnostic criteria concerning the core social phenotype of ASD, which emphasize difficulties with spontaneous self-initiated seeking of social encounters (that is, social motivation). Existing studies suggest that social 'wanting' tendencies vary considerably between individuals with ASD, and that the degree of social motivation is both malleable and predictive of intervention response. Although the topic of reward responsiveness in ASD is very new, with much research still needed, the current data clearly point towards problems with incentive-based motivation and learning, with clear and important implications for treatment. Given the reliance of behavioral interventions on reinforcement-based learning principles, the researchers believe that a systematic focus on the integrity of the reward system in ASD promises to yield many important clues, both to the underlying mechanisms causing ASD and to enhancing the efficacy of existing and new interventions.

4. Bloy, L., Ingalhalikar, M., Eavani, H., Schultz, R.T., Roberts, T.P.L., & Verma, R. (2012). White matter atlas generation using HARDI based automated parcellation. *Neuroimage, 59*(4), 4055-63.

Abstract. Most diffusion imaging studies have used subject registration to an atlas space for enhanced quantification of anatomy. However, standard diffusion tensor atlases lack information in regions of fiber crossing and are based on adult anatomy. The degree of error associated with applying these atlases to studies of children for example has not yet been estimated but may lead to suboptimal results. This paper describes a novel technique for generating population-specific high angular resolution diffusion imaging (HARDI)-based atlases consisting of labeled regions of homogenous white matter. Our approach uses a fiber orientation distribution (FOD) diffusion model and a data driven clustering algorithm. White matter regional labeling is achieved by our automated data driven clustering algorithm that has the potential to delineate white matter regions based on fiber complexity and orientation. The advantage of such an atlas is that it is study specific and more comprehensive in describing regions of white matter homogeneity as compared to standard anatomical atlases. We have applied this state of the art technique to a dataset consisting of adolescent and preadolescent children, creating one of the first examples of a HARDI-based atlas, thereby establishing the feasibility of the atlas creation framework. The white matter regions generated by our automated clustering algorithm have lower FOD variance than when compared to the regions created from a standard anatomical atlas.

5. Bloy, L., Ingalhalikar, M., Batmanghelich, N.K., Schultz, R.T., Roberts, T.P.L., & Verma, R. (2012). An integrated framework for HARDI-based investigation of structural connectivity. *Brain Connectivity, 2*(2), 69-79.

Abstract. Structural connectivity models hold great promise for expanding what is known about the ways information travels throughout the brain. The physiologic interpretability of structural connectivity models depends heavily on how the connections between regions are quantified. This article presents an integrated structural connectivity framework designed around such an interpretation. The framework provides three measures to characterize the structural connectivity of a subject: (1) the structural connectivity matrix describing the proportion of connections between pairs of nodes, (2) the nodal connection distribution (nCD) characterizing the proportion of connections that terminate in each node, and (3) the connection density image, which presents the density of connections as they traverse through white matter (WM). Individually, each possesses different information concerning the structural connectivity of the individual and could potentially be useful for a variety of tasks, ranging from characterizing and localizing group differences to identifying novel parcellations of the cortex. The efficiency of the proposed framework allows the determination of large structural connectivity networks, consisting of many small nodal regions, providing a more detailed description of a subject's connectivity. The nCD provides a gray matter contrast that can potentially aid in investigating local cytoarchitecture and connectivity. Similarly, the connection density images offer insight into the WM pathways, potentially identifying focal differences that affect a number of pathways. The reliability of these measures was established through a test/retest paradigm performed on nine subjects, while the utility of the method was evaluated through its applications to 20 diffusion datasets acquired from typically developing adolescents.

6. Bloy, L., Ingalhalikar, M., Eavani, H., Roberts, T., Schultz, R.T., Verma, R. (2011). HARDI based pattern classifiers for the identification of white matter pathologies. *Medical Image Computing and Computer Assisted Intervention*, *14*(Pt 2), 234-41.

Abstract. The paper presents a method for creating abnormality classifiers from high angular resolution diffusion imaging (HARDI) data. We utilized the fiber orientation distribution (FOD) diffusion model to represent the local WM architecture of each subject. The FOD images are then spatially normalized to a common template using a non-linear registration technique. Regions of homogeneous white matter architecture (ROIs) are determined by applying a parcellation algorithm to the population average FOD image. Orientation invariant features of each ROI's mean FOD are determined and concatenated into a feature vector to represent each subject. Principal component analysis (PCA) was used for dimensionality reduction and a linear support vector machine (SVM) classifier is trained on the PCA coefficients. The classifier assigns each test subject a probabilistic score indicating the likelihood of belonging to the patient group. The method was validated using a 5-fold validation scheme on a population containing autism spectrum disorder (ASD) patients and typically developing (TD) controls. A clear distinction between ASD patients and controls was obtained with 77% accuracy.

7. Ghanbari, Y., Herrington, J., Gur, R.C., Schultz, R.T., & Verma, R. (2013). Locality preserving non-negative basis learning with graph embedding. In J.C. Gee, S. Joshi, K.M. Pohl, W.M. Wells, & L. Zollei. (Eds.), *Information Processing in Medical Imaging 2013, Lecture Notes in Computer Science*, Vol. 7917, pp. 316–327. Springer, Heidelberg.

Abstract. The high dimensionality of connectivity networks necessitates the development of methods identifying the connectivity building blocks that not only characterize the patterns of brain pathology but also reveal representative population patterns. In this paper, we present a non-negative component analysis framework for learning localized and sparse sub-network patterns of connectivity matrices by decomposing them into two sets of discriminative and reconstructive bases. In order to obtain components that are designed towards extracting population differences, we exploit the geometry of the population by using a graph-theoretical scheme that imposes locality-preserving properties as well as maintaining the underlying distance between distant nodes in the original and the projected space. The effectiveness of the proposed framework is demonstrated by applying it to two clinical studies using connectivity matrices derived from DTI to study a population of subjects with ASD, as well as a developmental study of structural brain connectivity that extracts gender differences.

8. Ghanbari, Y., Smith, A, Schultz, R.T. Verma, R. (In press). Connectivity subnetwork learning for pathology and developmental variations, *Lecture Notes in Computer Science*.

Abstract. Network representation of brain connectivity has provided a novel means of investigating brain changes arising from pathology, development or aging. The high dimensionality of these networks demands methods that are not only able to extract the patterns that highlight these sources of variation, but describe them individually. In this pa- per, we present a unified framework for learning subnetwork patterns of connectivity by their projective non-negative decomposition into a re- constructive basis set, as well as, additional basis sets representing development and group discrimination. In order to obtain these components, we exploit the geometrical distribution of the population in the connectivity space by using a graph-theoretical scheme that imposes locality- preserving properties. In addition, the projection of the subject networks into the basis set provides a low dimensional representation of it that teases apart the different sources of variation in the sample, facilitating variation-specific statistical analysis. The proposed framework is applied to a study of diffusion-based connectivity in subjects with autism.

9. Tunc, B., Ghanbari, Y., Smith, A., Pandey, J., Browne, A., Schultz, R.T., & Verma, R. PUNCH: Population Characterization of Heterogeneity, In Preparation.

Abstract. Neuropsychiatric disorders are notoriously heterogeneous in their presentation, which precludes easy description and demonstration of the ways that these disorders differ from healthy controls and makes finding reliable biomarkers a challenge. There is a great need for reliable methods to capture underlying sample characteristics, particularly an overall severity measure of each dimension of functioning. This study proposes a general method of identifying and characterizing sample heterogeneity. The proposed framework identifies and quantifies the heterogeneity of any clinical population using a severity measure called PUNCH (Population Characterization of Heterogeneity) score. Researchers provide an exhaustive application of our framework to a simulated dataset and a large sample of youth with ASD taken from the CURE cohort, using phenotypic scores. The PUNCH scores from this population are used to create groups and study differences on DTI scans. Results demonstrate the ability of this new metric in

quantifying the underlying heterogeneity of the clinical samples, and suggest its utility in providing researchers with reliable severity assessments that can help parse population heterogeneity and improve statistical power to detect real differences from typically developing controls.

Background. Neurobehavioral disorders, such as autism spectrum disorder, are defined by difficulties in a number of discrete areas (e.g., social functioning, communication) that are, in principal, amendable to description along different severity dimensions. In fact, the National Institute of Mental Health (NIMH) has initiated a new funding mechanism to encourage researchers to move away from trying to map genetics and neurobiology onto diagnostic symptom descriptors and to instead focus on dimensions of behavior. Severity dimensions, the focus of this research, fit well within this new "Research Domain Criteria" (RDoC) approach by the NIMH, and should prove valuable in describing individual differences and correlating those with brain imaging data. However, finding reliable biomarkers to identify attributes of heterogeneous clinical populations is challenging because the boundaries between cases and typically developing controls (TDCs) is blurred with significant overlap for any given phenotypic feature. It is important to be able to aggregate phenotypic information into severity dimensions which allow better separation between groups and validation through independent measure of neurobiology (i.e., biomarkers). This process also improves the signal of the phenotypic information, creating a more reliable dimension capable of portraying clinical heterogeneity by the spread of the phenotypic measures under consideration. The aim of this study is to study the performance of a continuous severity measure obtained by combining various phenotypic scores with reference to independent brain imaging data. Details of this new method will be presented at the Performance Review. Figure 5.3 demonstrates results and utility of this approach.

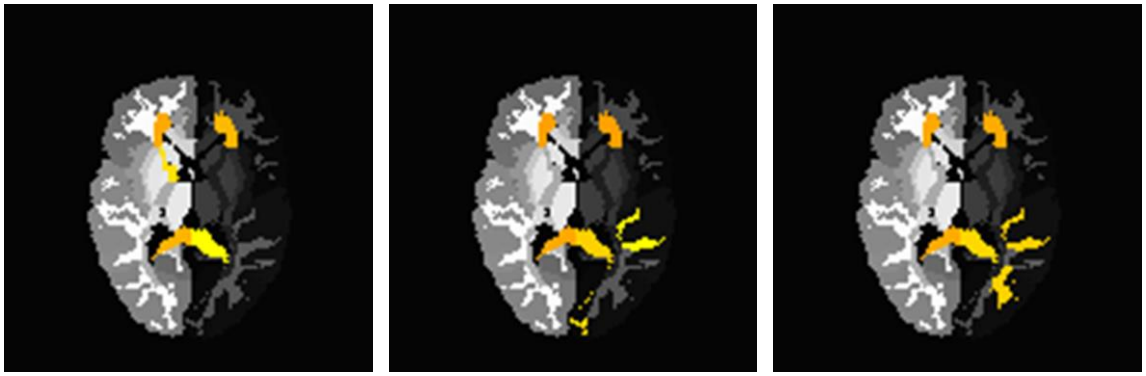


Figure 5.3. By defining subgroups of the ASD sample according to a continuous severity measure, differences between them and TDCs, which are not apparent when whole samples are used, can be revealed. This figure shows results of such study. The middle column of the figure above shows regions (out of 176 white matter regions of interest from an atlas) with significant FA differences between TDCs and the entire ASD. The third column shows more group differences, achieved by discarding the lower tail of the severity dimension within the ASD sample. Similarly, when the most severely affected portion of the ASD distribution is discarded (first column), some differences disappear.

Specific Aim 5.2: Progress

Specific Aim 5.2: To use fMRI and MEG to characterize social brain functioning differences in ASD that relate to genetic risk factors.

MEG Data Collection

MEG was collected from 150 subjects, using both an eyes-closed resting-state paradigm and a face identification task. Table 5.1 shows the number of participants in each group (collected and

Table 5.1

			Eyes Closed		Face Localization	
		Total Scanned	Collected	Evaluable	Collected	Evaluable
Controls	Males	65	64	47	65	59
	Females	6	6	6	6	6
		71	70	53	71	65
ASD	Males	63	63	41	61	49
	Females	9	9	7	9	8
		72	72	48	70	57
PDD	Males	5	5	2	3	2
	Females	2	2	1	2	1
		7	7	3	5	3
Total		**150**	**149**	**104**	**146**	**125**

evaluable) for these tasks. For eyes-closed, the number of evaluable data sets is reduced because not all participants kept their eyes closed throughout the resting exam. For the face identification task, the number of evaluable participants reflects only those whose behavioral measures showed they attended to the visual stimuli and performed the task.

Table 5.2

		Pure Tones		40Hz Steady-State	
		Collected	Evaluable	Collected	Evaluable
Controls	Males	64	62	58	49
	Females	5	5	3	2
		69	67	61	51
ASD	Males	53	52	46	37
	Females	6	6	6	4
		59	58	52	41
PDD	Males	3	3	2	1
	Females	1	1	1	0
		4	4	3	1
		132	**129**	**116**	**93**

In addition to resting-state and face localization data, researchers took advantage of the extra MEG scan time to pursue their interest in understanding auditory processes in ASD and MEG data was obtained for two additional tasks: (1) a pure tone auditory task, using 500 and 1000 Hz tones, and (2) a 40 Hz steady-state task. Table 5.2 shows the number of participants in each group for each auditory task. Numbers are slightly lower, as the researchers started collecting auditory data several months after beginning to collect the resting and face localization MEG data. Evaluable data indicates the number of subjects able to complete the task.

MEG Data Processing Pipeline (all tasks)
For all tasks (i.e., eye-closed, face identification, pure tone, 40 Hz steady-state), pre-processing analysis have been completed (i.e., correcting or removing artifact, co-registration to MRI, transformation to standard space for group analyses, calculating distributed source localization 4D maps). Resting-state, face localization, and auditory data are analyzed using a lead-field-based source localization method, Vector-based Spatio-temporal Analysis using L1-minimum norm (VESTAL; Huang et al 2006), to examine resting-state, face identification, and auditory processes throughout the brain. Figure 5.4 shows the steps for obtaining distributed source localization results.

MEG Resting-State Eyes-Closed Analyses
The researchers' first analyses examined whole-brain group differences in resting-state activity for delta (1-4 Hz), theta (4-8Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma activity (30+ Hz). Group differences were observed in delta and theta slowing (frontal regions), alpha (parietal-occipital areas), and beta and gamma activity (increased high-frequency activity in ASD throughout most of the brain).

For the resting exam, children were scanned in a seated position and were instructed to hold still with their eyes gently closed for 5 minutes. Electro-oculography data (EOG; bipolar oblique, upper right and lower left sites) were collected to ensure that participants' eyes remained closed throughout the 5-minute exam. After a band-pass filter (0.03–150 Hz), EOG and MEG signals were digitized at 1,000 Hz and down-sampled offline to 500 Hz.

MEG data were first processed with Temporal Signal Space Separation (TSSS; Taulu et al 2004) using Maxfilter (Elekta MaxfilterTM; Elekta Oy). TSSS separates neuronal magnetic signals arising from inside the MEG sensor array from external magnetic signals arising from the surrounding environment to reduce environmental noise and artifacts (e.g., magnetic artifacts due to metal objects, strong cardiac signals, environment noise, etc.). After TSSS, participants' raw EOG data were visually examined and epochs contaminated by blinks, saccades, or other significant EOG activity were removed. Any additional artifacts were rejected by amplitude and gradient criteria (amplitude 1,200 fT/cm, gradient 4,800 fT/cm/sample).

To coregister MEG and sMRI data, three anatomical landmarks (nasion and right and left preauriculars) as well as an additional 150+ points on the scalp and face were digitized for each subject using the Probe Position Identification (PPI) System (Polhemus, Colchester, VT). The three fiducials were identified in the subject's sMRI, and a transformation matrix that involved rotation and translation between the MEG and sMRI coordinate systems was obtained by matching the 150+ points from the PPI measurements to the surfaces of the scalp and face from the sMRI.

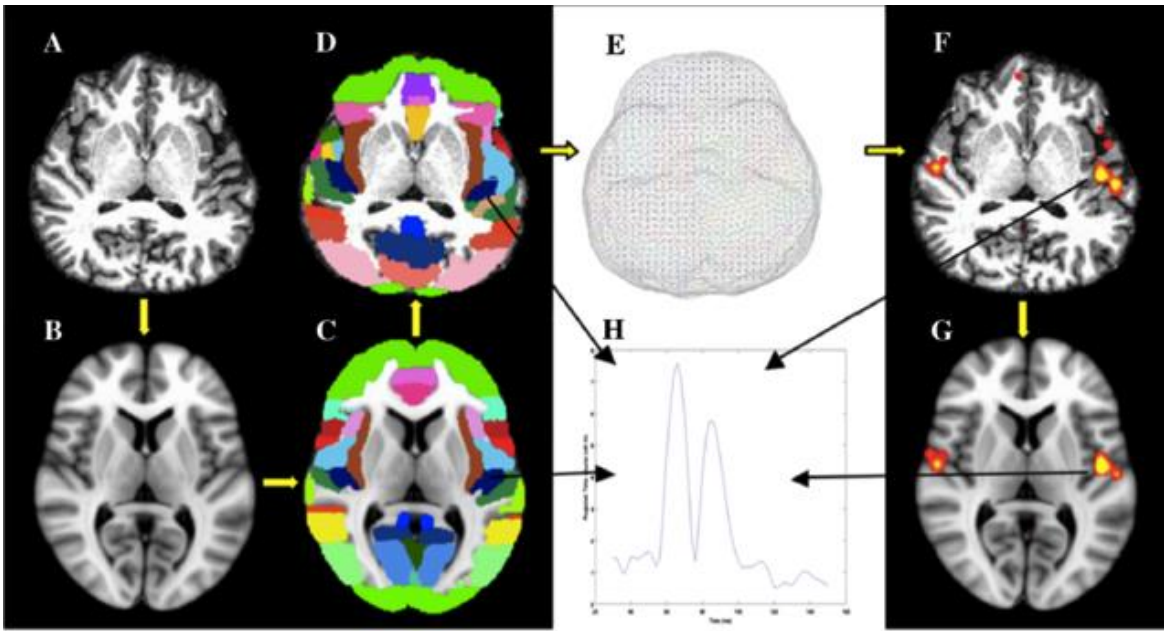


Figure 5.4. Distributed source localization analysis processing involves: (A) obtaining the T1-MRI from an individual subject; (B) MNI-152 Atlas space; (C) cortical mask fromMNI-152; (D) cortical mask transferred back to the individual MRI space; (E) VESTAL source grid with cortical and subcortical regions. Gray triangles are BEM mesh for MEG forward calculation; (F) VESTAL source image of the subject's auditory response overlaid on the T1-MRI; (G) VESTAL activity transferred to the MNI-152 coordinates; (H) regional time-course from VESTAL results. With the source images in standard space, within and between subjects analyses can be performed using standard imaging software (i.e., FSL, AFNI). As such, researchers are now able to quickly perform analyses examining differences in brain activity in TDC and the ASD children.

For VESTAL analyses, eyes-closed data were filtered to examine delta (1–4 Hz), theta activity (4–7 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-50 Hz). MEG data were divided into 2.5-second epochs with 50% overlap. For each epoch a Fast Fourier Transform (FFT) provided sensor-space FFT coefficients Kreal and Kimag. The sensor-space frequency-domain data were used by frequency-domain VESTAL to obtain the source amplitude (root mean squared) MEG source images. Additional details of frequency-domain VESTAL source imaging are provided in Huang et al. (2006).

Using the pipeline outlined in Figure 5.4, source images were obtained for each individual for each of the five frequency bands. To examine group differences throughout the whole brain, between-group t-tests compared VESTAL volumes. To obtain a family-wise corrected statistics map, a clustering method controlled family-wise error. The clustering method computes the probability of a random field of noise producing a cluster of adjacent time-frequency cells of a given size after the noise is thresholded at a given probability level, thereby providing a corrected p-value. The cluster size needed to obtain the desired family-wise correction was determined using a standard fMRI package (AFNI AlphaSim, B. Douglas Ward, 2000). Results are provided only for alpha activity.

To the researchers' knowledge, this is the first study to use whole-brain MEG analyses to assess resting alpha activity in children. The primary generators of alpha activity were localized to parietal-occipital regions. In particular, as shown in Figure 5.5, areas with the greatest alpha activity for ASD (shown in blue) and for TDC (shown in red) were observed in midline parietal-occipital areas. A surprising finding was that group differences were not observed in primary alpha regions. Rather, as shown in the green overlays, significantly increased alpha activity in ASD was observed in regions where little alpha activity was observed in TDC.

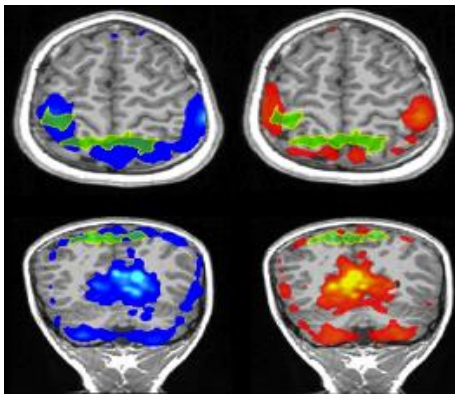


Figure 5.5

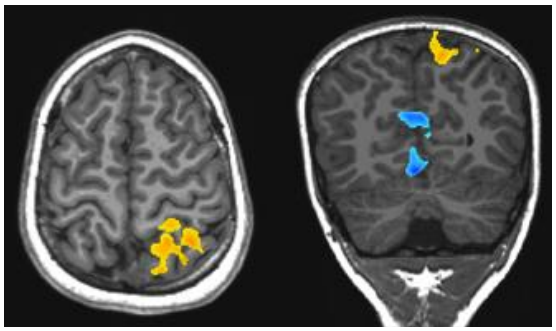


Figure 5.6

Furthermore, increased alpha activity was associated with increased symptoms of ASD. Family-wise corrected statistics maps showing associations between alpha activity and SRS scores are shown in Figure 5.6. Children with ASD with the greatest alpha activity in regions outside the primary alpha regions had the highest SRS scores (yellow). In contrast, children with ASD with the greatest activity in primary alpha regions had the lowest SRS scores (blue).

The alpha findings provide insight into potential mechanisms and functional correlates of atypical alpha activity in ASD. Alpha is inversely related to perception and attention, suggesting that alpha reflects functional inhibition of sensory systems (Jokisch et al 2007). It is possible that increased alpha power at rest renders the ASD brain unprepared for sensory input and thus may be associated with sensory processing abnormalities in ASD, such as delayed auditory M100 evoked responses (Roberts et al 2010) and deficient rapid temporal auditory processing (Cardy et al 2005). Futures work directly comparing resting alpha activity and sensory processing in children with ASD would address this hypothesis. Second, inhibitory interneurons, which are likely abnormal in ASD (Cardy et al 2005; Levitt et al 2004) play a role in maintaining alpha oscillations (Lorincz et al 2009), suggesting a potential mechanism for the observed alpha findings. Interestingly, interneuron abnormalities in a mouse model of ASD are specific to frontal and parietal regions (Levitt et al 2005), demonstrating similar regional specificity to the delta and alpha findings reported in the present study. In sum, the resting-state alpha findings demonstrate atypical resting-state oscillatory activity in children with ASD, suggest that regionally- and spectrally-specific alpha anomalies are of possible clinical relevance, and support an imbalance of neural excitation/inhibition as a potential ASD biomarker.

A manuscript reporting the alpha findings will be submitted to a special ASD issue of *Brain*

Connectivity. A paper reporting activity in the other frequency bands, examining changes in resting oscillatory activity as a function of age in ASD and TDC is also being prepared. Moreover, resting-state findings provided pilot data for a R21 awarded July of 2013 to CURE co-investigator Dr. J. Christopher Edgar (5R21MH098204-02; Functional connectivity in autism spectrum disorders). The goal of the R21 is to use the advanced source localization methods developing as part of CURE study to examine an emerging but mostly untested hypothesis that the symptoms of autism are due to impaired functional connectivity (FC) within and between brain regions. In addition to the above alpha findings, the researchers have observed focal differences in resting and auditory task neural activity between ASD and TDC. As described above, these focal irregularities (e.g., increased posterior alpha power) have clinical implications and are associated with ASD symptoms. The R21 uses these findings as a starting point to examine neural oscillatory connectivity abnormalities in ASD. In particular, using our resting-state and auditory findings to determine ROIs, the R21 tests the hypothesis that brain activity in ASD is associated with atypical local cross-frequency coupling (CFC) and long-range FC.

In addition to the frequency band analyses, the researchers also measured the latency to peak signal response during the face-processing task in 40 participants with ASD and 44 TDCs (see **Table 5.3**)

Table 5.3. Participant Characteristics.

	ASD mean (SD)	TDC mean (SD)	p-value
Age	10.1 (1.9)	9.7(1.9)	0.306
IQ (GCA)	108.3(22.7)	111.9(14.6)	0.406
SRS T-total	76.1(11.4)	40.4(5.3)	<0.01
SCQ	21.4(6.1)	1.8(1.9)	<0.01
Benton	35.3(4.2)	38.5(4.7)	0.01

MEG was collected following presentation of 50 upright faces and 50 upright houses using the Elekta MEG system. Each face and house stimulus was repeated 4 times for 500ms, with a 1600ms or 2400ms inter-stimulus interval. Participants were given the opportunity to rest during a resting slide if needed. Data were motion, artifact and blink corrected and averaged using the program BESA. The latency of the peak between 100 and 140 ms was picked to represent the visual M100 response.

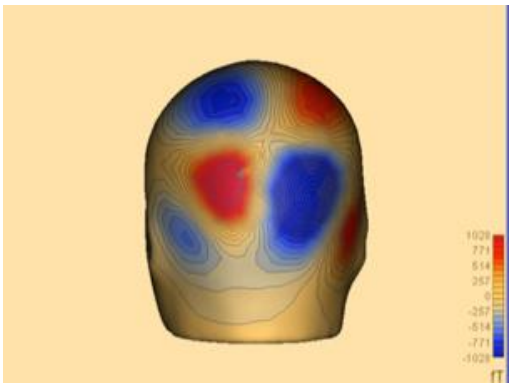


Figure 5.7

An example of the 100ms visual response in occipital cortex is shown in Figure 5.7. The latency of the peak in the temporal fusiform face area between 140 and 300ms that follows the M100 response was picked to represent the M170 component. The M170 had separate right and left hemisphere components.

Results. The latencies of the 100ms visual response to faces and houses were analyzed using a multivariate ANOVA covaried with age (SPSS). A main effect of group was found for faces ($F(2, 83) = 4.1$, $p=0.02$) but not houses ($F(2, 83) = 1.6$, $p=0.2$) for the 100ms component. Specifically, ASD subjects had a 5 ms delay to faces compared to TDC (Bonferroni-corrected $p=0.02$), but no delay for houses ($p=0.9$) (Figure 5.8).

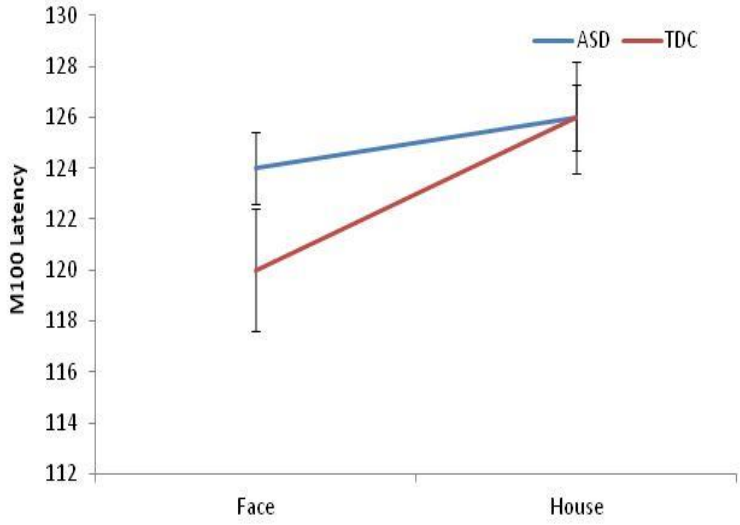


Figure 5.8

The researchers have previously demonstrated latency delays to faces with event related potentials (McPartland et al, 2011), at the N170 peak. However, no group differences were found in the M170 component in the current study. The researchers have previously demonstrated a ~10 ms latency delay to auditory tones in a separate cohort of ASD. Thus, a final analysis examined the correlation between the M100 face latency and the M100 latency to auditory tones to see if the latency delays are a global sensory phenomenon (i.e. subjects with auditory delays

also have visual delays). However, the partial correlation between the visual 100ms latency and the auditory 100ms latency, correcting for age, was not significant (r = -0.09).

Finally, the researchers describe results from a recent MEG publication (Ghanbari, Y., Bloy, L., Edgar, J.C., Blaskey, L., Verma, R., Roberts, T. Joint Analysis of Band-Specific Functional Connectivity and Signal Complexity in Autism. Journal of Autism and Developmental Disorders, In Press.)

Examination of resting state brain activity using electrophysiological measures like complexity as well as functional connectivity is of growing interest in the study of autism spectrum disorders (ASD). The present paper jointly examined complexity and connectivity to obtain a more detailed characterization of resting state brain activity in ASD. Multi-scale entropy was computed to quantify the signal complexity, and synchronization likelihood was used to evaluate functional connectivity (FC), with node strength values providing a sensor-level measure of connectivity to facilitate comparisons with complexity. Sensor level analysis of complexity and connectivity was performed at different frequency bands computed from resting state MEG from 26 children with ASD and 22 typically developing controls (TD). Analyses revealed band-specific group differences in each measure that agreed with other functional studies in fMRI and EEG: higher complexity in TD than ASD, in frontal regions in the delta band and occipital-parietal regions in the alpha band, and lower complexity than TD in delta (parietal regions), theta (central and temporal regions) and gamma (frontal-central boundary regions); increased short-range connectivity in ASD in the frontal lobe in the delta band and long-range connectivity in the temporal, parietal and occipital lobes in the alpha band. Finally, and perhaps most strikingly, group differences between ASD and TD in complexity and FC appear spatially complementary, such that where FC was elevated in ASD, complexity was reduced (and vice versa). The correlation of regional average complexity and connectivity node strength with symptom severity scores of ASD subjects supported the overall complementarity (with opposing sign) of connectivity and complexity measures, pointing to either diminished connectivity leading to elevated entropy due to poor inhibitory regulation or chaotic signals prohibiting effective measure of connectivity.

fMRI Studies: Progress

As described in the original application, fMRI studies involved three face processing tasks: 1) passive viewing of individual faces, 2) passive viewing of dynamic facial expressions, and 3) subordinate level judgment (same/different identify) of two neutral faces presented side-by-side. All tasks follow a block design (20-sec 5-trial blocks and 12 sec rest), alternating same/different judgments of houses (tasks 1, 2, and 3) with passive viewing of faces (tasks 1 and 2) or same/different judgments of faces (task 3). FMRI scan parameters were the same for all runs (3.5 mm isotropic voxels, TR=2340 ms, TE = 25 ms, 140 repetitions).

In addition, the researchers had proposed an *exploratory fMRI* resting state study, requiring participants to relax and look at a fixation cross during for the duration of the run (with identical scanning parameters). In a second run, researchers had proposed performing a continuous fMRI "steady state" task after Hampson et al. (2002) that required participants to watch a continuous set of movies of children playing. Movies were of two types: (a) parallel play, where the two

child actors played side by side but did not engage in any social communication and (b) joint play, involving two children playing together with a shared game, and significant amounts of social communication (gesture, facial expressions, but there was no sound or talking since that would be hard to hear during the MRI). Participants were asked to complete a quiz about the content of the videos at the end of each run to check if they were paying adequate attention to the information presented. The scan parameters are the same as in the primary fMRI Task except more images were acquired (210 instead of 140, lasting 8.19 sec).

Face Perception fMRI Data. As already noted, 164 children were scanned with MRI as part of Project 5. However, evaluable face perception data across the three tasks types was only available for 159 participants, including 76 with ASD (63 male, 12 female; mean age = 10.3 years, ± 1.62) and 83 TDC (69 male, 14 female; mean age = 9.8 years, ± 1.80). Mean DAS II GCA (IQ) scores were 103.5 (±21.7) for the ASD group, and 112.6 (±13.5) for the TDC group. Across all 159 participants, very little individual run data was lost after quality control procedures for movement (> 1.5 mm absolute displacement or half a voxel) and other image artifacts. Five run A (passive viewing of individual faces), six run B (same/different identify of two neutral faces presented side-by-side) and two run C (passive viewing of dynamic facial expressions) were excluded from further analyses.

The researchers were able to collect addition runs of face perception data by tacking one or more of these 3 runs onto other funded studies. Including these data brings the total number of unique participants for runs A, B and C to 272, 271 and 275, respectively, for final analyses. This is the largest fMRI ASD dataset known to these researchers and because DNA was also collected, it provides an excellent opportunity to examine genotypic X endophenotypic associations. Moreover, researchers will further test the utility of PUNCH (Tunc et al, in preparation), the phenotypic severity metric describe at the end of the progress report for Aim 5.1.
FMRI data were processed using the FMRIB Software Library (FSL; Jenkinson and Smith, 2004). All fMRI data were spatially (FWHM = 4mm) and temporally filtered (1/60 Hz high-pass). Outlier signal intensities (spikes) were removed using the AFNI program 3DDespike (Cox, 1996). FMRI data were registered to standard space (MNI) using affine transformations. First-level (time series) analyses followed a general linear model (GLM) where activity in each voxel was predicted by parameters reflecting each task type (i.e., faces, houses, and rest periods). As quality control measures are not complete on the full data set at the time of this report, preliminary results are not presented. However, the current approach tests for group differences using a mixed-model ANOVA on all voxels (including the random effect of participant within group, i.e., a "random effects" analysis). Planned task and group comparisons will be conducted as *t*-tests. Connectivity analysis will focus primarily on psychophysiological interactions – multiple regression, where activity of each intra-cerebral voxel is predicted by the interaction of signal within a seed region (e.g., fusiform gyrus, a key face processing area) and the task parameter (e.g., faces vs. houses). In many neuroimaging studies, stronger conclusions can be reached regarding task-relevant brain functions if they are proven independent from particular visual scanning patterns. Our MRI head coil is equipped with Avotec Real Eye/SMI eye tracking system. Although eye tracking data were not a critical dependent variable in this proposal, we collected these data during fMRI scanning on the majority of participants and will integrate them into the analyses. Signal within fMRI clusters can be compared (via GLM) to a variety of basic eye-tracking metrics, such as mean time spent fixating on eyes versus mouth,

number of saccades between face regions, etc.

As described above with respect to the DTI data, the researchers have created a new severity algorithm (Tunc et al, in preparation) that shows promise in helping understand the heterogeneity inherent in ASD, and in particular to help identify milder cases, and will allow testing of how clinical severity moderates group differences. After completing the more standard group level analyses, next steps will be to use Multivariate Psycho-Physiological Interactions for evaluation of functional and informational connectivity (as has been pioneered by colleagues at UPenn, Coutanche et al). The researchers' prior work on correlating ASD symptom severity with fMRI signals has shown that multi-voxel activity patterns contain information that cannot be extracted from univariate statistical procedures (Coutanche, Thompson-Schill, Schultz, 2011).

Steady State Social Perception Task. Results from this paradigm have been presented recently at the International Meeting for Autism Research (Giovannelli, et al, 2013, IMFAR, San Sebastian, Spain). The final data set is comprised of 139 participants (the researchers discontinued using this task in the magnet about 6 months prior to the end of the fourth year of funding, so as to be able to collect eye tracking data on all participants outside of the magnet for the eye tracking comparison study described in Project 4: McVey et al, submitted poster). fMRI results presented at IMFAR and below concern a sample of 82: 34 ASD (9 female) and 48 TDC (15 female). Methods. This task entailed a steady state block design with two conditions (as already described): videos of children playing together (joint) or playing separately (parallel). Eye tracking data were collected during fMRI scanning (Avotec RealEye System, Sensori-Motoric Instruments). Figure 5.9 shows examples of the video stimuli captured as 2 still frames with one participants gaze pattern and fixation (blue circles) superimposed. Participants were instructed to watch the videos and stay as still as possible. After the fMRI task, participants completed a surprise memory game to test attention to videos. As with other components of the fMRI study, data were collected at 3T, across 40 axial slices (voxel resolution = $3.5^{3;}$; TR = 2340 ms; TE = 25 ms; number of volumes = 210).

Figure 5.9. Screenshots of videos for the joint (right) and parallel (left) play conditions with eye tracking data overlay (in blue). Circles on the data overlay represent fixations and scale in size with fixation time.

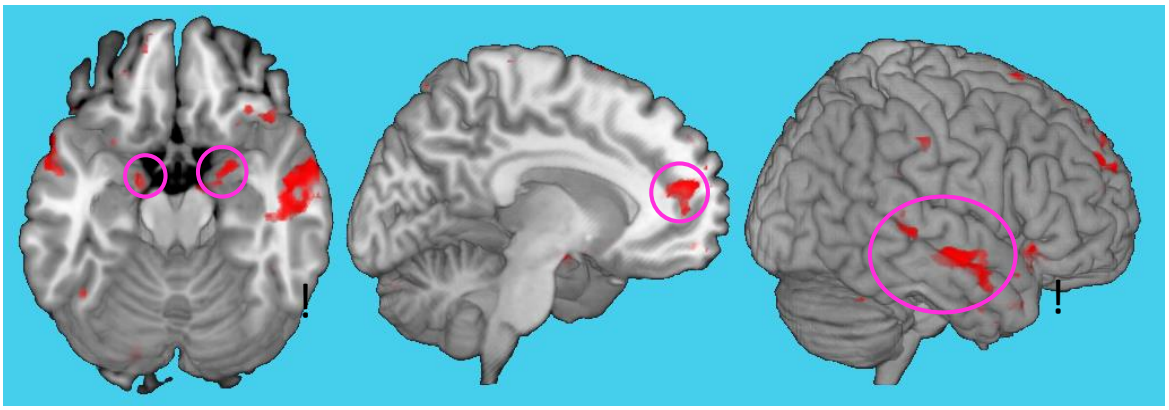
Per-Voxel analyses of Task and Group Effects involved a mixed model ANOVA, with separate explanatory variables representing joint and parallel play conditions that were convolved with double-gamma hemodynamic response function. Family-wise error correction ($p < .05$) was implemented via cluster size thresholding, using the same general procedures as described above

for MEG (VESTAL) data. Additionally, fMRI data for this task were examined using independent component analyses (ICA, implemented via FSL's program MELODIC). ICA analyses examined independent spatio-temporal signal patterns across time, simultaneously for all participants. However, these components could be decomposed into task (joint vs. parallel) and group (ASD vs. TDC)-specific effects.

Results: Per-voxel Analyses: For the per-voxel joint versus parallel play contrast, the ASD group showed decreased activation in amygdala, STS, and medial PFC (see Table 5.4 and Figure 5.10). Right fusiform face area (FFA) was increased for TDCs, but group difference did not survive family wise error correction; this difference might be expected as both movie types contained the same amount of face identity information, for which the fusiform gyrus is selective.

Table 5.4: Regions showing greater activation for TDC vs. ASD for the contrast joint > parallel.

Region	**Center of Gravity (MNI)**	**Peak Z**	**Cluster Size (2mm Voxels)**
Anterior Cingulate/ Medial Frontal Gyrus	2,52,27	3.87	590
Right STS	55,-8,-16	3.90	777
Left Posterior STS	-48, -45, 1	3.62	140
Right Amygdala	20,-2,-17	3.46	66
Left Amygdala	-17,-5,-17	3.78	33



AMYGDALA Medial PFC STS

Figure 5.10. Regions showing greater activation for TDC vs. ASD for the per voxel contrast of joint > parallel play ($p < .05$, corrected)

Results: ICA. A single independent component

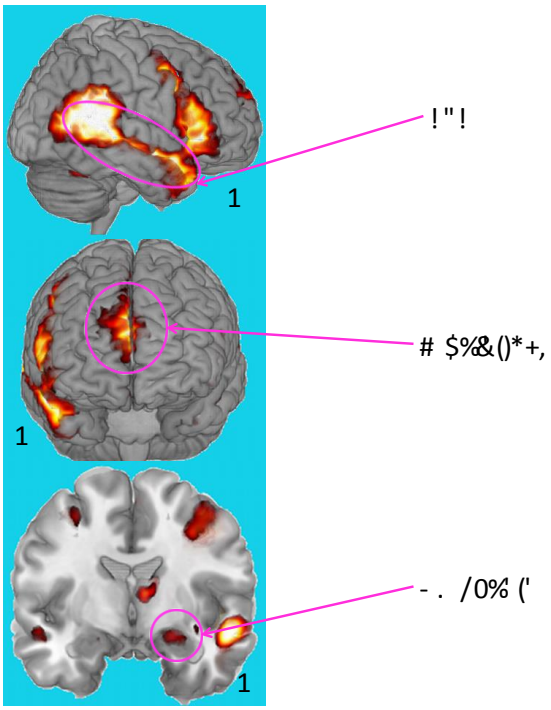


Figure 5.11. ICA group comparison (TDC>ASD) at p<.05 (corrected).

(IC) was isolated that encompassed 3 of 4 hypothesized areas (superior temporal sulcus [STS], amygdala and medial prefrontal gyrus) for the joint vs. parallel contrast ($p < .001$). The TDC group had significantly greater weightings on this component compared to the ASD group ($p = .003$), confirming the results of the GLM per voxel analyses (See Figure 5.11). Moreover, these spatio-temporal ICA analyses revealed abnormal connectivity across the social brain, and were specific to social perception (joint versus parallel play). Results were replicated using a subset of participants that were group matched for time looking at faces (eye tracking; data not shown). Thus, brain based ASD hypoactivation cannot be attributed to abnormal eye gaze patterns. These results suggest that naturalistic social stimuli may be particularly well suited to eliciting deficits in network connectivity in ASD (vs. TDC).

Resting State Functional Connectivity. Results from a subset of resting state data have been presented recently at the International Meeting for Autism Research (Bartley, et al, 2013, IMFAR, San Sebastian, Spain). The fMRI results presented at IMFAR focused on the functional separations of components responsible for social processes, and abnormalities in these components in the ASD sample. The final dataset for future analyses contains 166 participants (77 ASD; 89 TDC).

Methods. Subjects were instructed by researchers to focus their attention on a stationary crosshair for the duration of the resting state scan (~ 6 minutes). FMRI analyses were conducted using the same analysis pipeline as described above (i.e., FSL). However, resting state fMRI analyses requires additional preprocessing steps – particularly related to participant head motion. The influence of motion was controlled by removing volumes in the time series that displayed framewise displacements greater than 0.5 mm (Power et al., 2012). Cerebral spinal fluid (CSF) and white matter (WM) segmentations of structural data for each subject were performed using

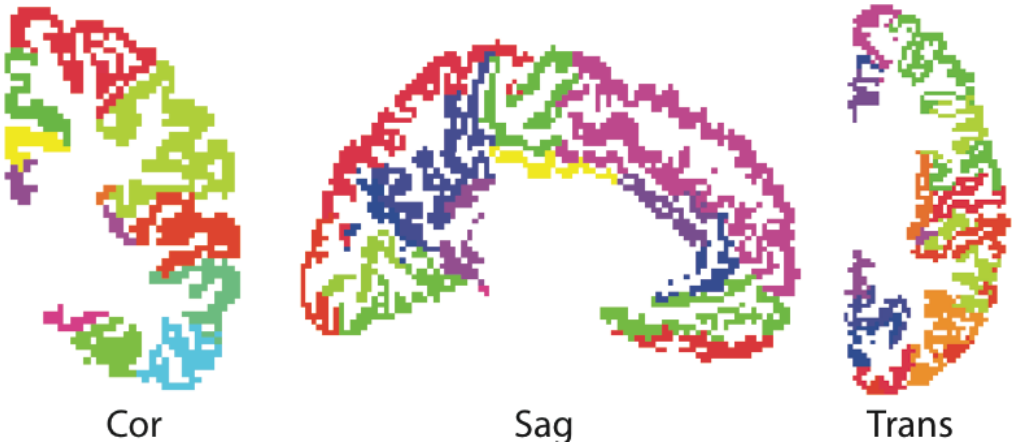


Figure 5.12. Cortical parcellations from Desikan-Killiany Freesurfer atlas.

FSL's program FAST, and eroded 1 voxel using FSL. Single value decomposition (SVD) was performed across voxels within these regions of noninterest and voxels representing the top 2% of standard deviations over the course of the run. Resulting top 6 SVD time series were regressed out of the time-series and band-passed filtered between 0.01 and 0.09 Hz. Cortical parcellations of regions of interest were performed using the Desikan-Killiany Freesurfer atlas (Desikan et al., 2006; see Figure 5.12) and FSL's program FIRST.

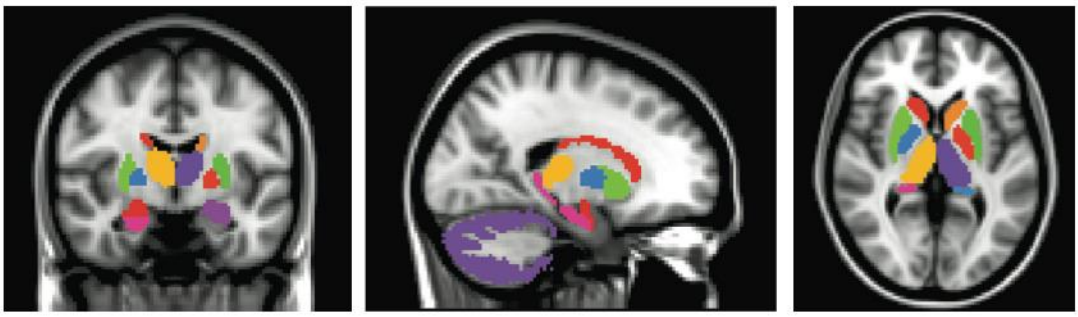


Figure 5.13. Subcortical parcellations from FSL FAST.

Results: Using residuals from the nuisance regression measures, severity of repetitive behaviors (measured by the Repetitive Behaviors Scale-Revised; RBS-R) were correlated with measurements of connectivity strength (as represented by bivariate correlations of time series fluctuations in activity) between parcellation-defined regions of interest. Using this approach the researchers found that time-series fluctuations in the cerebellum, post-central, and pre-central gyri within subjects assessed correlated with severity of repetitive behavior, suggesting that these circuits contribute to repetitive motor behavior in ASD.

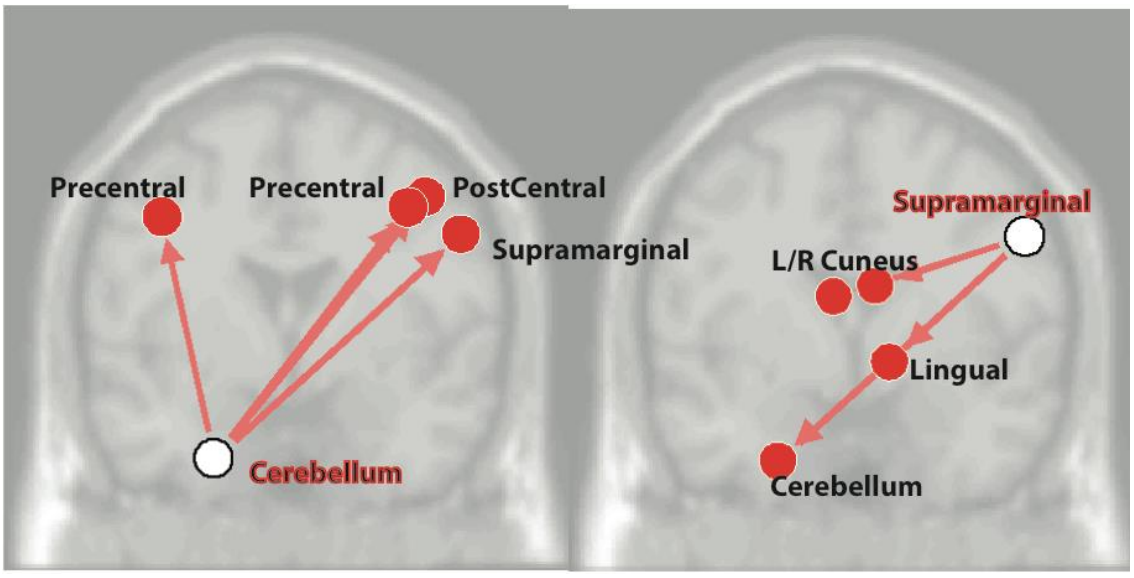


Figure 5.14. Positive correlations between RBS-R symptom severity and connectivity strength in ASD subjects (p<.05, corrected).

Ongoing analyses are taking advantage of the increased statistical power present in using the entire resting state sample to see if this will reveal other correlational differences in cortical and subcortical connectivity with clinical measures of symptom severity within ASD. Following emerging developments in fMRI connectivity analyses (i.e., Rubinov & Sporns, 2010), the researchers' approach is has begun to draw from graph theory, which is specifically designed to characterize the behavior of networks (including networks of brain areas). The integration of the researchers' newly developed severity metric (Tunc et al., in preparation) will greatly enhance these analyses.

Specific Aim 5.3: We will use multivariate pattern classification to establish whether aggregating fMRI, sMRI, DTI, and MEG data can improve our ability to robustly categorize individual brains as characteristic of ASD or TDC.

Analyses of the CURE data for Aim 3 cannot begin until unimodal data analyses are closer to completion. Since data collection was just finished, unimodal analyses remain the focus. The researchers expect to make significant progress on Aim 5.3 after unimodal analyses are complete.

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PROJECT 6: MINORITY TRAINING PROGRAM (PI: JAMES CONNELL, PhD)

Project 6 Specific Aims:

Project 6 had one Specific Aim:

Specific Aim 6.1: Develop a Program in Autism Research Training (PART) for high-achieving undergraduate and post baccalaureate minority students who express an interest in pursuing a career in clinical services or science related to the field of autism.

Aggressive strategies are necessary if we are to expand the pool of minority applicants to doctoral-level research training programs. This is a critical mission in light of the poor representation of certain minorities in the field of autism research and clinical services. To address this problem, Project 6 created two training programs, one for undergraduate students and one for post-baccalaureate/graduate students. Students were recruited through a variety of avenues, including Leadership in Neurodevelopmental Disorders (LEND), McNair Scholars program, PennPREP, Temple University, and Lincoln University, as well as the University of Pennsylvania.

PART was initially led by Peter Doehring, PhD, CAR's Director of Clinical Training. Dr. Doehring has extensive experience developing and implementing ASD training programs. The PI of Project 6 was changed to Dr. James Connell in January of 2011 when Dr. Doehring left CHOP. Dr. Connell had been on the faculty at Temple University, and is an experienced clinician and interventionist. In his new role, Dr. Connell increased the media and print recruitment efforts at Lincoln and Temple Universities, and the University of Pennsylvania by developing an e-flyer which was circulated through university student association listservs (e.g., psychology student council, Psi Chi, psychology honors program), and printed for posting on psychology program bulletin boards. Dr. Connell visited the local universities and described to students directly, the aims of the CURE grant of the minority training program. Dr. Connell coordinated with Dr. Nicole Stevens at Lincoln University, the Project's primary partner. Dr. Stevens assisted in advertising this opportunity to under-represented minority students at Lincoln, and mentored students who joined PART. Dr. Connell also met individually with local university students, corresponded with national students via email and phone, and met via Skype with interested international students. As a result of these targeted and aggressive recruiting efforts, the remaining undergrad summer positions were filled for the 2011 summer session. The remaining two post-baccalaureate positions were filled in the fall of 2011.

PART successfully met its training goals and recruited 4 undergraduate and 4 post-baccalaureate students for this program (per the original proposal), each of whom is a member of an underrepresented group. Every student met the criteria outlined in the original proposal (e.g., having an undergraduate GPA of at least 3.0, and submission of a written application describing their interest and future scientific goals).

The undergraduate program successfully recruited 4 scholars - 2 part time scholars who spent the academic year on their project, working at least 10 hours per week, and 2 full time summer scholars, as planned in the original grant application. Students were matched with a faculty mentor based on their research interests, and some had a secondary mentor, reflecting the breadth of the student's interests, the multidisciplinary nature of CAR and the field of autism research. Two undergraduate students completed their program with Dr. Nicole Stephens at Lincoln University and two at CAR under the mentorship of Dr. Connell and colleagues. Each was paid a stipend as described in the original application.

The post- baccalaureate also successfully recruited 4 scholars. These received a more intensive mentored experience, as they had a two-year full time job to prepare them for competitive graduate programs. After completing the program, each of the post-baccalaureate minority students was successful in gaining entrance to graduate school or to full time employment with a position directly related to autism.

- One is currently in her third year in a PhD program at the University of Florida studying Clinical Psychology.
- A second begins a PhD program in Clinical Psychology at Washington University, St. Louis in August of 2013.
- A third is in an applied behavior analysis master's program and is focusing on autism interventions in community settings.
- The fourth is working full time at the Autism Science Foundation in New York City.

Each undergraduate and post - baccalaureate student had clinical exposure (for some this was more extensive), designed to stimulate their interest in clinical research during medical school and post-graduate training. Mentors ensured that their students became familiar with community-based supports, service providers, and advocacy organizations. In addition, mentors met regularly (formally at least once per week) with their students, and during the course of the student research project, they were mentored about design, conduct, and the evaluation of clinical research. Many students interacted nearly daily with their mentors and researchers at CAR since the nature of the research and lab space encourages face-to-face time. Their research experience also entailed learning more about issues of data collection and study management, and ethical issues regarding human subjects use in clinical research. Post- baccalaureate students presented their work at one or more scientific meetings (e.g., CAR's full Center Science Meeting, the LEND research day, and in one case at a national meeting [the American Psychological Association, where the student won the runner up prize for best student research]).

Each student received a wide array of autism research training, including attending workshops, attending Grand Rounds, Case Conferences, CAR's Distinguished Lecture Series, the Next Steps trainings for parents of newly diagnosed children with autism and Next Steps into Adolescence trainings. In addition, student scholars were mentored on interviewing and applying to graduate school by members of CAR, their primary mentor and in some cases through PennPREP (an established post-Baccalaureate Scholars program at the University of Pennsylvania) and/or the McNair Scholars program.

CARs Distinguished Lecture Series: Attendance at CAR's Distinguished Lecture Series (DLS) was expected for all students (and attending at least 4 of the lectures was required). The DLS features some of the most prominent autism researchers and clinicians in the world. Topics range from immunology to genetics to behavioral interventions. PART students were expected to discuss with Dr. Connell, their lab supervisor, and/or peers and colleagues the content of the presentation, questions they might have about the content or the area of research, and if this type of research was something they would like to learn more about. The purpose of this exercise was to encourage the students to think about career options, areas of interest and how they might pursue that area of interest in graduate training programs. See the addendum at the end of this section for a listing of DLS speakers during the funding period for the CURE grant.

"Next Steps" workshops. Students were required to attend three workships, one of each of the 3 kinds (see below). Workshops focused on the developmental changes that occur during childhood and adolescence for children on the spectrum. Workshops featured presentations from experts in psychology, behavior health, medicine, social work, and education. The students discussed what they learned with Dr. Connell, their PART peers, and/or clinical staff following the presentation. They were encouraged to ask questions about what they learned, and consider areas of interest to consider as future research/clinical careers. The workshops were originally created about 6 years by clinicians and social workers at CAR for parents of newly diagnosed children, as they were faced with lots of questions about "next steps". With the great feedback received from the community and success of the original workshop, CAR clinicians lead by Gail Stein, MSW, created two additional workshops, one aimed at professionals (especially those new to the field, since they often gained entrance into the original workshop, which was not its intended audience) and one aimed at caregivers of pre-adolescent youth with ASD who were

transitioning to adolescents.

Next Steps Workshop for Families of Young Children. This workshop is for parents of young children newly diagnosed with autism spectrum disorders (ASD). Topics include an overview of ASD and accompanying conditions, available therapies and treatments, how to decide what interventions to pursue, and tips for supporting families living with ASD. Presenters include specialists in developmental and behavioral pediatrics, speech and language development, occupational therapy, psychology, and special education law. Parents who have made the next steps with their children will participate in a family panel discussion.

Next Steps Workshop for Professionals. This is a workshop for professionals working with young children newly diagnosed with autism spectrum disorder (ASD). Topics include an overview of ASDs and accompanying conditions, available therapies and treatments, how to decide what interventions to pursue, and tips for supporting families living with ASD. Two researchers from CAR will present their studies. Other presenters include specialists in developmental and behavioral pediatrics, speech and language development, occupational therapy, and social work. Parents who have made the next steps with their children will participate in a panel led group discussion.

Next Steps into Adolescence. This workshop is for parents of a pre-adolescent or adolescent child with an autism spectrum disorder (ASD). As children approach and enter the teen years,

they must deal with physical growth spurts, mindboggling hormone releases, and ever-changing and challenging social relationships. For pre-adolescent children with ASD, these already complicated physical and social changes are compounded by the typical ASD-difficulties of reading social cues and understanding the behaviors of others. Consequently, children with ASD approaching adolescence have greater transition needs than most typically developing pre-teens. Likewise, parents can benefit from understanding and knowing what they might expect as their children progress through this next developmental stage. The workshop features presentations from experts in psychology, behavior health, medicine, social work, and education. Parents who have adolescent children with ASD participate in a panel led group discussion.

Case conferences. PART Students were required to attend at least one CAR case conference, which were held two times per month when the Center began, and now are held once per month. Case conference presentations are attended by 10-15 clinical faculty and clinical postdocs working in CAR's research clinic and in CHOP's outpatient ASD clinics in psychiatry and child development. Presentations focused on interesting and/or complex cases that the presenting clinician felt were valuable for teaching or for group discussion to assist with their own formulation. During these presentations, the lead clinician for that week used a seminar format to present cases. Frequently readings that might assist with the discussion were distributed ahead of the meeting. Junior clinicians were expected to offer assessment recommendations with rationale for the tools suggested, discuss the potential differential diagnosis, and/or discuss evidence-based treatment recommendations related to the identified behavioral domain deficits. PART students were expected to benefit from the case conferences by becoming familiar with the assessment process, and the complex case conceptualization that frequently occurs when assessing children on the spectrum.

LEND autism course: This course (6 2-hour didactics) was designed to prepare individuals from a variety of disciplines to become knowledgeable ASD, and was taught as part of the Leadership Education in Neurodevelopmental Disabilities (LEND) program through CHOP. The LEND program trains individuals from 13 different health and education disciplines around neurodevelopmental disabilities services, clinical work, and research. Topics for this autism course included the characteristics of autism spectrum disorders and the diagnostic terms used; the impact of ASD on the family; case management and the burden parents often experience in caring for their child and navigating the services available to them; the history of ASD and the role of science, parent groups, and the media; epidemiological studies of the rate of ASD; the role of screening across a wide age range, the role of the interdisciplinary team in evaluation and treatment; evidence-based interventions; applied behavioral analysis; differentiating between ASDs and other psychiatric, behavioral, and medical conditions; and interdisciplinary assessment. Didactic instructors represent the fields of developmental pediatrics, psychology, school psychology, and social work. In addition to these didactics, each participant observed two evaluations at the interdisciplinary ASD Assessment Clinic in Child Developments Regional Autism Clinic.

Additional training happened as part of an autism seminar by CAR faculty that was specifically geared to these trainees and others at their level of education. These seminars covered a range of topics including: an introduction to autism (autism 101), assessment and classification, the biological underpinnings of autism, intervention and evidence based practices. Drs. Doehring

and Connell used informal discussion to provide an overview of these topics and met monthly with each of the post-bac PART students to follow-up with questions/topics that arose in previous presentations and discussions.

Finally, students were exposed to the assessment and intervention processes by observing in the CAR assessment clinic, and in the local public school system where autism specific interventions are implemented. Several of the students had specific roles in the clinic or school system based projects, but those that did not were required to attend and observe several case assessments. The assessment clinic at CAR uses a variety of IQ, performance and neuropsychological testing instruments. Students learned about these instruments, what they purport to represent, and the rationale for assessment tool selection. The PART students then observed the assessments and met with the clinicians to discuss the process. The PART students also completed field observations of autism intervention implementation in public school classrooms. The students shadowed Dr. Connell's consultants as they provided direct support, in-class training and didactic presentation of autism interventions to public school teachers.

Project 6 Addendum: DLS speakers across the 4 years of CURE funding.

2009-2013 CAR Distinguished Lecture Series

Date	Speaker	Topic
September 24, 2009	Richard Paylor, Ph.D. Professor, Department of Molecular and Human Genetics, Professor, Department of Neuroscience, Baylor College of Medicine	Autistic-like Traits in Mouse Genetic Models
October 22, 2009	W. Ian Lipkin, M.D. John Snow Professor of Epidemiology, Professor of neurology and Pathology, Mailman School of Public Health and College of Physicians and Surgeons, Columbia University	Microbiology and Immunology of Autism and Neurodevelopmental Disorders
November 12, 2009	Joseph Buxbaum, M.Sc., Ph.D. Professor of Psychiatry, Neuroscience, Genetics and Genomic Sciences, Mount Sinai School of Medicine	Rare Genetic Variants in Autism and Related Conditions
November 19, 2009	Ricardo Dolmetsch, Ph.D. Assistant Professor, Neurobiology, Stanford University	From Calcium Channels to Autism

December 3, 2009	Laura Schreibman, Ph.D. Distinguished Professor of Psychology, Director of the Autism Intervention Research Program, University of California, San Diego	One Side Does Not Fit All: Research in Refining and Individualizing Behavioral treatment of Autism
January 21, 2010	Larry J. Young, Ph.D. William P. Timmie Professor, Dept. of Psychiatry, Emory University School of Medicine	Molecular Neurobiology of Social Cognition and Bonding: Implications for Autism Spectrum Disorders
February 10, 2010	Marshalyn Yeargin-Allsopp, M.D. Chief, Developmental Disabilities Branch, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention	Autism: What's New and What's to Do
February 11, 2010	Marshalyn Yeargin-Allsopp, M.D. Chief, Developmental Disabilities Branch, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention	Epidemiology and the Changing Paradigm of ASD
March 4, 2010	Janine LaSalle, Ph.D. Professor, Medical Microbiology and Immunology, Rowe Program in Human Genetics, University of California, Davis	15q11-14 Chromatin Dynamics in Autism Spectrum Disorders
March 24, 2010	Evdokia Anagnostou, M.D. Clinician Scientist, Bloorview Research Institute Assistant Professor, Department of Pediatrics, University of Toronto	What We Know and Don't Know About Medications in Autism
March 25, 2010	Evdokia Anagnostou, M.D. Clinician Scientist, Bloorview Research Institute Assistant Professor, Department of Pediatrics, University of Toronto	Translational Approaches to Psychopharmacology of Autism: The Oxytocin Example
April 1, 2010	James C. McPartland, Ph.D. Associate Research Scientist and Associate Director, Developmental Electrophysiology Laboratory, Yale Child Study Center	A matter of Time: Electrophysiological Studies of Social Perception
April 8, 2010	David Amaral, Ph.D. Professor, Dept. of Psychiatry and Behavioral Sciences Director of Research, UC Davis M.I.N.D. Institute	Dealing with the Heterogeneity of Autism: The Autism Phenome Project
April 22, 2010	Lonnie Zwaigenbaum, M.Sc., M.D. Co-director, Autism Research Centre, Glenrose Rehabilitation Hospital and Associate Professor, Department of Pediatrics, University of Alberta	Early Developmental Trajectories in ASD: Insights from Studies of High-Risk Infants

April 22, 2010	Lonnie Zwaigenbaum, M.Sc., M.D. Co-director, Autism Research Centre, Glenrose Rehabilitation Hospital and Associate Professor, Department of Pediatrics, University of Alberta	Early Detection of Autism: It Takes a Community
April 29, 2010	Randy Blakely, Ph.D. Allan D. Bass Professor of Pharmacology and Psychiatry, Vanderbilt University Director, Center for Molecular Neuroscience, Vanderbilt/NIMH Silvio O. Conte Center for Basic Neuroscience Research	Too Much of a Good Thing: Autism, the Serotonin Transporter and Its Regulatory Genes
May 27, 2010	Tristram H. Smith, Ph.D. Associate Professor, Department of Pediatrics, University of Rochester, School of Medicine and Dentistry	Applied behavior Analysis for Children with Autism Spectrum Disorders: State of the Science
May 27, 2010	Tristram H. Smith, Ph.D. Associate Professor, Department of Pediatrics, University of Rochester, School of Medicine and Dentistry	Early Intensive Behavioral Intervention for Children with Autism Spectrum Disorders: What Families and Providers Need to Know
September 8, 2010	Matthew S. Goodwin, Ph.D. Director of Clinical Research at the MIT Media Lab and Associate Director of Research at the Groden Center	Developing Innovative Technologies for Autism Research & Practice
September 15, 2010	Connie Kasari, Ph.D. Professor, Psychological Studies in Education and Psychiatry, University of California, Los Angeles	Peer Interactions and Engagement of Children with ASD in General Education Classrooms
September 16, 2010	Connie Kasari, Ph.D. Professor, Psychological Studies in Education and Psychiatry, University of California, Los Angeles	Early Intervention for Children with Autism: Why Targeted Treatment on Core Deficits is Important
September 16, 2010	Connie Kasari, Ph.D. Professor, Psychological Studies in Education and Psychiatry, University of California, Los Angeles	Optimizing Communication Outcomes in Infants at Risk for Autism
September 28, 2010	Vince Calhoun, Ph.D. Director, Image Analysis and MR Research, The Mind Research Network Associate Professor, Dept. of Electrical and Computer Engineering Neurosciences and Computer Science, University of New Mexico	ICA-based Analyses and Simulation of Multimodal Brain Imaging and Genetic Datasets

September 29, 2010	Edwin Cook, Jr., M.D. Director, Center for Neurodevelopmental Disorders, Professor of Psychiatry, University of Illinois at Chicago	Autism: A Paradigmatic Complex Genetic Disorder
November 11, 2010	Jeffrey Wood, Ph.D. Assistant Professor, Psychological Studies in Education and Psychiatry, University of California, Los Angeles	Cognitive Behavioral Approaches to Addressing Core Autism Symptoms in School Ages Children
November 11, 2010	Jeffrey Wood, Ph.D. Assistant Professor, Psychological Studies in Education and Psychiatry, University of California, Los Angeles	Understanding and Addressing Anxiety in Youth on Autism Spectrum
January 12, 2011	Bryan King, M.D. Director, Psychiatry and Behavioral Medicine; Program Director, Seattle Children's Autism Center	Carrying Coals to Newcastle: An Update on the Pharmacotherapy of Hyperactive and Repetitive Behaviors in ASD
January 13, 2011	Bryan King, M.D. Director, Psychiatry and Behavioral Medicine; Program Director, Seattle Children's Autism Center	Accomplishments in the Science Community's Search for Autism Causes and Effective Treatments and Pressing Challenges in the Path Forward
March 10, 2011	Lisa Croen, Ph.D. Senior Research Scientist, Division of Research Director, Autism Research Program, Kaiser Permanente Northern California	Epidemiologic approaches to investigating immune system involvement in autism
March 10, 2011	Lisa Croen, Ph.D. Senior Research Scientist, Division of Research Director, Autism Research Program, Kaiser Permanente Northern California	What Causes Autism? An Epidemiologist's Approach to Searching for Clues
April 6, 2011	Marshalyn Yeargin-Allsopp, M.D. Chief, Developmental Disabilities Branch, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention	What's New and What to Do
April 7, 2011	Marshalyn Yeargin-Allsopp, M.D. Chief, Developmental Disabilities Branch, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention	Epidemiology and the Changing Paradigm of ASD
April 28, 2011	Sally Rogers, Ph.D. Professor, Psychiatry and Behavioral Sciences,	Early Detection of ASD and Implications of Earliest Treatment

	UC Davis M.I.N.D. Institute	
April 28, 2011	Sally Rogers, Ph.D. Professor, Psychiatry and Behavioral Sciences, UC Davis M.I.N.D. Institute	Can we Identify and Treat Autism in Infancy
June 9, 2011	Mark Strauss, Ph.D. Director, Infant and Toddler Development Center, Professor of Psychology, University of Pittsburg	The importance of implicit cognitive process to understanding autism; categorization and the development of facial knowledge
September 1, 2011	Marsha Mailick Seltzer, Ph.D. Director, WaismanCenter; Professor, Brandeis University	Impacts of autism on the family
September 22, 2011	Peter Mundy, Ph.D. Director of Educational Research, U.C. Davis MIND Institute	Education and social issues for school-aged children
October 20, 2011	Deborah Fein, Ph.D. Professor, Departments of Psychology and Pediatrics, University of Connecticut	Early intervention therapies
November 17, 2011	Eric Fombonne, M.D. Professor of Psychiatry, McGill University; Director, Autism Spectrum Disorders Program, Montreal Children's Hospital	Epidemiological approaches to understanding the causes of autism
February 2, 2012	Timothy Roberts, Ph.D. Vice Chair of Research, Department of Radiology, The Children's Hospital of Philadelphia	The MEG study: How the brain processes words, sounds, and pictures and the resulting impact
March 21, 2012	Peter Marshall, Ph.D. Associate Professor, Department of Psychology, Temple University	Early social and cognitive development
April 12, 2012	Ami Klin, Ph.D. Chief of Autism and Related Disorders, Department of Pediatrics, Emory University School of Medicine	The social mind
April 26, 2012	Shana Nichols, Ph.D. Director, ASPIRE Center for Learning and Development	Autism and Girls
May 31, 2012	Peter Szatmari, Ph.D. Professor, Vice-Chair of Research, Head of Division of Child Psychiatry, McMaster University	The Emergence of Heterogeneity in ASD

May 31, 2012	Peter Szatmari, Ph.D. Professor, Vice-Chair of Research, Head of Division of Child Psychiatry, McMaster University	Adult Outcome Studies in ASD: Lessons for Early Diagnosis and Intervention
October 4, 2012	Susan Levy, MD Director, Regional Autism Center; Medical Director, Center for Autism, CHOP Judith Miller, PhD, Director of CAR Clinical Training, CHOP Gail Stein, LCSW, CAR Social Worker CHOP	Autism and the DSM: History, Current and Future Criteria, and Implications
November 8, 2012	John Herrington, PhD Associate Director of the Developmental Neuroimaging Laboratory, Center for Autism Research, CHOP Martin Franklin, PhD, Director, Child and Adolescent OCD, Tic, Trich & Anxiety Group (COTTAGe), University of Pennsylvania	Anxiety and Autism Spectrum Disorders
December 13, 2012	Clarence E. Schutt, PhD Director & Chief Scientific Officer, Nancy Lurie Marks (NLM) Family Foundation	A 20 year Perspective on Trying to Understand Autism
December 13, 2012	Clarence E. Schutt, PhD Director & Chief Scientific Officer, Nancy Lurie Marks (NLM) Family Foundation	A Structural Biologist Looks at Autism: What is it?
January 17, 2013	Jennifer Pinto-Martin, PhD, MPH Viola MacInnes/ Independence Professor of Nursing, Chair, Department of Biobehavioral Health Sciences, University of Pennsylvania	Autism: Epidemiology and Etiology
February 7, 2013	Robert Schultz, PhD Director, Center for Autism Research, CHOP	Using fMRI to Study the Brain
February 21, 2013	Timothy Roberts, PhD Vice Chair of Research, Department of Radiology, The Children's Hospital of Philadelphia	The Brain and Gamma Synchrony, Excitation/Inhibition, and Translational Research
March 7, 2013	Sarah Paterson, PhD Director of the Infant Neuroimaging Lab, Center for Autism Research, CHOP	Infant and Toddler Research in Autism Spectrum Disorders
April 4, 2013	David Mandell, ScD Director, Center for Mental Health Policy & Services Research,	New Findings on Implementing Evidence-Based Practices for Students with Autism in

	University of Pennsylvania Perelman School of Medicine; Associate Director, Center for Autism Research, The Children's Hospital of Philadelphia	Public Schools

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

__X__Yes ***(Project 4)***
_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

__X__Yes ***(Project 4)***
_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

__16*__Number of hospital and health care professionals involved in the research project
*those who conducted diagnostic testing & clinical phenotyping were counted here

18(D) How many subjects were included in the study compared to targeted goals?

_480__Number of subjects originally targeted to be included in the study
_678__Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_591__Males
__87__Females
___0__Unknown

Ethnicity:
__48__Latinos or Hispanics
_577__Not Latinos or Hispanics
__53__Unknown

Race:
___1__American Indian or Alaska Native
__12__Asian
__91__Blacks or African American
___0__Native Hawaiian or Other Pacific Islander
_522__White
__35__Other, specify:Biracial (33); North African Arab (1); Kazakh (1)
__17__Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

Philadelphia and Montgomery counties only.

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. The impact of the metabotropic glutamate receptor and other gene family interaction networks on the autism spectrum disorders. ***(Project 1)***	D. Hadley, Z. Wu, C. Kao, A. Kini, A. Mohamed-Hadley, K. Thomas, L. Vazquez, H. Qiu, F. Mentch, R. Pellegrino, C. Kim, AGP Consortium, J. Glessner, & H. Hakonarson	Molecular Psychiatry	June 2013	☑Submitted ☐Accepted ☐Published
2. The role of mGluR network genes in genetic and environmental forms of syndromic autism spectrum disorder. ***(Project 1)***	T.L. Wenger, C. Kao, D.M. McDonald-McGinn, E.H. Zackai, A. Bailey, R.T. Schultz, B.E. Morrow, B.S. Emanuel, & H. Hakonarson	JAMA	June 2013	☑Submitted ☐Accepted ☐Published

3. Whole-genome sequencing in an autism multiplex family. ***(Project 1)***	L. Shi, X. Zhang, R. Golhar, F.G. Otieno, M. He, C. Hou, C. Kim, B. Keating, G.J. Lyon, K. Wang, & H. Hakonarson	Molecular Autism	October 2012	☐Submitted ☐Accepted ☑Published
4. From mouse to human: Evolutionary genomics analysis of human orthologs of essential genes. ***(Project 2)***	B. Georgi., B. Voight, & M. Bucan	PLOS Genetics	December 2012	☐Submitted ☐Accepted ☑Published
5. The basolateral amygdala (BLA) is activated during social approach and investigation in juvenile C57BL/6J mice. ***(Project 3)***	A.S. Kreibich, M. Torre, C.T. Piccoli, H. Li, R. Gur, T. Abel, E. Brodkin	Journal of Neurosci-ence	February 2013	☑Submitted ☐Accepted ☐Published
6. Salient social cues are prioritized in autism spectrum disorders despite overall decrease in social attention. ***(Project 4)***	C. Chevallier, P. Huguet, F. Happé, N. George, & L. Conty.	Journal of Autism and Develop-mental Disorders	October 2012	☐Submitted ☑Accepted ☐Published
7. Susceptibility to the audience effect explains performance gap between children with and without autism in a Theory of Mind task. ***(Project 4)***	C. Chevallier, J. Parish-Morris, N. Tonge, L. Le, J. Miller, & R.T. Schultz	Journal of Experimen-tal Psychology: General	June 2013	☑Submitted ☐Accepted ☐Published
8. A Signal Detection Approach to Quantifying Social Motivation: Developing Tools for the Research Domain Criteria Framework. ***(Project 4)***	C. Chevallier, N. Tonge, V. Troiani, G. Kohls, J. Miller, & R.T. Schultz	American Journal of Psychiatry	June 2013	☑Submitted ☐Accepted ☐Published
9. Social rejection enhances preconscious	C. Chevallier,* V. Troiani,* (*co-first authors), & R.T. Schultz	Cognition	February 2013	☑Submitted ☐Accepted ☐Published

processing of faces. ***(Project 4)***				
10. Children with autism do not show sequence effects with auditory stimuli. ***(Project 4)***	C. Molesworth, C. Chevallier, & F. Happé	Journal of Experimen-tal Psychology: General	July 2013	☑Submitted ☐Accepted ☐Published
11. Levels of autistic traits in Anorexia Nervosa: a comparative psychometric study. ***(Project 4)***	A. Courty, A.S. Maria, C. Lalanne, D. Ringuenet, C. Vindreau, C. Chevallier, L. Pouga, F. Pinabel, A. Philippe, J.L. Adrien, C. Barry, & S. Berthoz	BMC Psychiatry	May 2013	☑Submitted ☐Accepted ☐Published
12. Social 'wanting' dysfunction in autism: neurobiological underpinnings and treatment implications. ***(Projects 4 & 5)***	G. Kohls, C. Chevallier, V. Troiani, & R.T. Schultz	Journal of Neurodevel-opmental Disorders	March 2012	☐Submitted ☐Accepted ☑Published
13. Visual attention to dynamic faces and objects is linked to face processing skills: A combined study of children with autism and controls. ***(Project 4)***	J. Parish-Morris,, C. Chevallier, N. Tonge, J. Letzen, J. Pandey, & R.T. Schultz	Frontiers in Psychology	February 2012	☐Submitted ☐Accepted ☑Published
14. The social motivation theory of autism. ***(Projects 4 & 5)***	C. Chevallier, G. Kohls, V. Troiani, E.S. Brodkin, & R.T. Schultz	Trends in Cognitive Science	January 2012	☐Submitted ☐Accepted ☑Published
15. Reward associations modulate awareness of novel objects. ***(Project 4)***	V. Troiani, C. Chevallier, & R.T. Schultz	Psychologi-cal Science	December 2012	☑Submitted ☐Accepted ☐Published
16. The broad autism phenotype predicts child Functioning in autism spectrum disorders. ***(Project 4)***	C.R. Maxwell, J. Parish-Morris, O. Hsin, J.C. Bush, & R.T. Schultz	Journal of Neurodevel-opmental Disorders	March 2013	☑Submitted ☐Accepted ☐Published
17. White matter atlas generation	L. Bloy, M. Ingalhalikar, H. Eavani, R.T. Schultz,	Neuroimage	June 2011	☐Submitted ☐Accepted

using HARDI based automated parcellation. ***(Project 5)***	T.P.L. Roberts, & R. Verma			☑Published
18. An integrated framework for HARDI-based investigation of structural connectivity. ***(Project 5)***	L. Bloy, M. Ingalhalikar, N.K. Batmanghelich, R.T. Schultz, T.P.L. Roberts, & R. Verma	Brain Connectivity	January 2011	☐Submitted ☐Accepted ☑Published
19. Locality preserving non-negative basis learning with graph embedding. ***(Project 5)***	Y. Ghanbari, J. Herrington, R.C. Gur, R.T. Schultz, & R. Verma	Information Processing in Medical Imaging 2013, Lecture Notes in Computer Science	March 2012	☐Submitted ☐Accepted ☑Published
20. Connectivity subnetwork learning for pathology and developmental variations. ***(Project 5)***	Y. Ghanbari, A. Smith, R.T. Schultz, & R. Verma	Lecture Notes in Computer Science	February 2013	☐Submitted ☐Accepted ☑Published
2. Joint analysis of band-specific functional connectivity and signal complexity in autism. ***(Project 5)***	Y. Ghanbari, L. Bloy, J.C. Edgar, L. Blaskey, R. Verma, & T. Roberts	Journal of Autism and Develop-mental Disorders	April 2013	☐Submitted ☐Accepted ☑Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X____ No_____

If yes, please describe your plans:

Project 1:
The researchers have an article in preparation entitled, "Novel sequence variants in *CDH26* associate with autism spectrum disorder". Here we are reporting on the SNP association observed with *CDH26* in autism, and further annotating the functional impact of these SNPs as well as discussing the other variants we uncovered in the 100 cadherin/protocadherin genes tested.

The researchers also have an article in preparation including results from the autism family trios we sequenced.

Project 2:
A publication describing the method for the annotation of regulatory variants and the application to ASD candidate genes will be submitted by September 2013:
Wadhawan S., Choi I., Nguyen N., Brown C., Won K.J. Bucan M., (2013), Systematic identification of regulatory sequences from brain epigenetic marks, in preparation.

At least two additional publications will describe a full spectrum of rare variants found in ASD subjects (including all collected with CURE funding as well as those collected from related non-PA grants).

Project 3:
The researchers anticipate writing and submitting at least 2 manuscripts on the *Pcdh10*+/- mouse model in the coming year.

Projects 4 & 5:
In response to Question 17, the researchers described numerous studies that are currently in progress and that will be submitted for peer review and publication. It is difficult to put a precise estimate on the number of future publications, but clearly it will be a dozen or more.

Project 6:
None.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Project 1:
The impact from the research conducted has led to the discovery of novel sequence variants that explain additional heritability of autism and may have therapeutic implications.

Project 3:
None because this project was conducted in mouse models. However, the research is likely to lead to translational applications to patient care in the future, including informing possible biomarkers and pharmacologic treatments for ASD.

Projects 2, 4 & 5:

The scientific findings from Projects 2, 4, & 5 have not had a direct impact on "the incidence of disease, death from disease, stage of disease at time of diagnosis" because (a) ASD is not life threatening and (b) the research proposed and funded did not concern early detection of ASD (which could have had an impact on "stage of disease at time of diagnosis").

However, the scientific findings from the CURE grant will have a demonstrable impact on the scientific community's understanding of the clinical features of ASD, how best to parse the clinical heterogeneity, and the underlying brain mechanism that are the proximal cause of ASD. The CURE grant generated one of the largest clinical research databases on ASD known to exist, and will support many scientific discoveries.

Project 6:
The scientific findings from the CURE grant have not had a direct impact on "the incidence of disease, death from disease, stage of disease at time of diagnosis" because (a) ASD is not life threatening and (b) the research proposed and funded did not concern early detection of ASD (which could have had an impact on "stage of disease at time of diagnosis"). However, this project supported numerous students who are beginning their careers in autism research and who might have otherwise taken a different career path.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Projects 1, 2, 4, 5, & 6:
None.

Project 3:
We have identified a novel mouse model of ASD, the Pcdh10+/- mouse model. This model can be used in the future to elucidate the neurobiology and pathophysiology underlying behavioral symptoms of ASD. Also, the model can be used to test novel treatments for ASD. We have already identified a pharmacologic agent—d-cycloserine—that can restore sociability in this mouse model.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes___X____ No_____

If yes, please describe your plans:

Project 1:
We plan to file on *CDH26* discovery reported.

Projects 2-6:
None.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Biosketches for all key investigators were included in the original grant application with the exception of James Connell, PhD. Please find his biosketch attached.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Connell, James Edward	Clinical Director and Research Fellow, AJ Drexel Autism Institute
eRA COMMONS USER NAME: JECLL2	

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Temple University, Philadelphia, PA.	BA	1997	Psychology
Louisiana State University	MA	2003	School Psychology
Louisiana State University	PhD	2005	School Psychology
May Institute (APA Internship)		2004	Clinical Psychology

A. Personal Statement

I am a practicing psychologist, a nationally certified school psychologist and a board certified behavior analyst conducting mental health, behavioral health and educational research in community settings. For the past 10 years, my scholarship and clinical practice has focused on the conceptualization and implementation of academic and behavioral health interventions, and the development and refinement of consultant-driven procedures to ensure program fidelity. I have more than 15 years experience working in school systems and in mental health settings providing consultative support to direct care staff and educators regarding the implementation of evidence-based interventions. I have published numerous studies identifying external variables associated with the adult behavior change needed to ensure successful program implementation and have critiqued and edited numerous peer-reviewed papers on the same topic. I have taught graduate level courses in academic and behavioral interventions, and school-based consultation models and procedures. My research and clinical goals are to advance the dissemination and implementation of evidence-based interventions in community settings.

B. Positions and Honors

Positions and Employment

2004-2005 APA Clinical Intern, May Institute, Newton MA.
2005-2007 School Psychologists, Salem City Public Schools, Salem NJ
2007-2010 Assistant Professor, School Psychology Program, Psychological Studies in Education, Temple University, College of Education
2007-2010 Affiliated Faculty, Applied Behavior Analysis Program, Curriculum, Instruction and Technology in Education, Temple University, College of Education
2010- 2012 Research Associate, Department of Psychiatry, University of Pennsylvania School of Medicine
2010- 2012 Research Associate, Center for Autism Research, The Children's Hospital of Philadelphia
2012- Clinical Director and Research Fellow, AJ Drexel Autism Institute; Associate Professor of Education, Drexel University

C. Selected peer-reviewed publications (abbreviated).

1) Day J & Connell JE. (2013). Establishing a generalized repertoire of helping behavior in adolescents with autism: A replication. *Journal of Applied Behavior Analysis. (in print).*
2) Pisacreta,J., Tincani, M., Connell, JE., & Axelrod, S. (2011). Increasing teachers' Use of a 1:1 Praise to behavior Correction Ratio to Decrease Student Disruption in General Education Classrooms. Behavioral Interventions, 26, 243-339.
3) Pellecchia, M., Connell, J.E., Eisenhart, D., Kane, M., Schoener, C., Turkel, K., Riley, M., & Mandell, D. S. (2011). Group Performance Feedback: Consultation to Increase Classroom Team Data Collection, Journal of School Psychology, 49, 411-431.
4) Hinchey M, & Connell JE (2010). A Quantitative analysis of language interventions for children with autism. The Behavior Analyst Today, 11,128-144.

5) Connell JE (2010). Applications of performance feedback: Consultation in the home. International Journal of Behavioral Consultation and Therapy, 6, 17-23.
6) Codding R, Archer J, & Connell JE (2010). A Systematic Replication and extension using incremental rehearsal to improve multiplication skills: An investigation of generalization. Journal of Behavioral Education, 19, 93-105.
7) Cabellero A, & Connell JE (2010). Evaluation of the effects of social cue cards for preschool age children with Autism Spectrum Disorders (ASD). Journal of Behavior Assessment and Intervention in Children, 1, 25-42.
8) Handler MH, Rey J, Connell JE, Thier K, & Putnam B (2007). Practical considerations in creating school-wide positive behavior support in public schools. Psychology in the Schools, 44, 29-40.
9) Noell GH, Witt JC, Slider NJ, Connell JE, Williams KL, Resetar JL, & Koenig JL (2005). Treatment implementation following behavioral consultation in schools: A comparison of three follow-up strategies. School Psychology Review, 34, 87-106.
10) Ardoin SP, Witt JC, Suldo SM, Connell JE, Koenig JL, Resetar JL, Slider NJ, & Williams KL (2004). Examining the incremental benefits of administering a MAZE and three versus one curriculum-based measurement reading probes when conducting universal screening. School Psychology Review, 33, 118-133.
11) Noell GH, Duhon GJ, Gatti SL, & Connell JE (2002). Consultation, follow-up, and implementation of behavior management interventions in general education. School Psychology Review, 31, 217-234.
12) Ringdahl J, Vollmer T, Borrero J, & Connell JE (2001). Fixed-time schedule effects as a function of baseline reinforcement rate. Journal of Applied Behavior Analysis, 34, 1-16.

D. Research Support

(RFA 02-11) (Lawer, L, PI) 07/01/11 – 06/30/14
Commonwealth of Pennsylvania
Autism Services, Education, Resources & Training Center – Eastern Region
The purpose of the ASERT centers, which are funded by Pennsylvania Department of Public Welfare, Bureau of Autism Services, is to address the needs of Pennsylvanians with autism across the Commonwealth. The ASERT Centers enhance the lives of Pennsylvanians with autism across the age span by bringing together resources to improve regional access to quality services and supports, train professionals in best practices and facilitate collaboration among providers of services throughout the Commonwealth.
Role: Co-Investigator

(Connell, JC, PI) 07/01/11 – 06/30/13
Millville School District
Philly AIMS Consultation
This clinical contract provides valuable consultation and coaching to the Millville School District administrative leadership core, building level administration and classroom staff on the implementation of response-to-intervention and positive behavioral interventions and supports system-wide initiatives. Under Dr. Connell's supervision, three post-docs working in 6 elementary schools are leading the systemic change needed to implement evidence-based practices related to the effective classroom instruction and behavioral support. Under the supervision of Dr. Connell, coaches are evaluating many of the variables associated with program fidelity and meeting to discuss dissemination and implementation models for future research projects.