Genes, brain, and behavior: development gone awry in autism?

A report on the 23rd Annual International Symposium of the Center for the Study of Gene Structure and Function

Michael J. Lewis1,a and Jason B. Dictenberg2,a

Departments of 1Psychology and 2Biological Sciences, Hunter College of the City University of New York, New York, New York

Addresses for correspondence: Michael J. Lewis, Department of Psychology, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065. mlewis@genectr.hunter.cuny.edu; Jason B. Dictenberg, Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065. dictenberg@genectr.hunter.cuny.edu

Autism and its highly variable symptomology were the themes of the 23rd Annual International Symposium of the Center for the Study of Gene Structure and Function at Hunter College in New York City, held 15 January 2010. The meeting explored the extensive research on autism from several perspectives—integrating research on genetics, neuroscience, and behavior—from researchers presenting new and innovative approaches to understanding the autism spectrum. Early diagnosis, intervention, and genetics were major themes because they are seen as essential areas in which progress is needed before the rise in numbers of cases of autism throughout the world, which some describe as approaching an epidemic, can be stemmed. Several genetic, neurobiological, and behavioral markers of autism have been identified that may ultimately provide the basis for early identification, and that presently define the key areas requiring intensive intervention.

Keywords: autism spectrum disorder (ASD); neuron; synapse; epilepsy; endophenotype; fragile X syndrome (FXS); microcephaly with seizures (MCSZ); mental retardation; connectome; brain circuit; axon; dendrite; Early Start Denver Model (ESDM); mirror neurons; Asperger’s syndrome; specific language impairment (SLI); joint attention

Autism—Integrating Genes, Brain and Behavior, was a day-long symposium held at Hunter College of the City University of New York. It was the 23rd Annual International Symposium of Hunter’s Center for the Study of Gene Structure and Function and was cosponsored by the Clinical and Translational Science Center at Weill Cornell Medical College. The symposium provided an exciting exploration of the basic research on the molecular genetics and neurobiological mechanisms of autism, as well as the social and cognitive research on autism. The speakers, in their respective fields, shared an appreciation for the complexity of autism and the challenges of understanding it. Each speaker offered a unique approach to these challenges using a wide range of research tools and skills. The research and analysis covered ranged from molecular biology and real-time neuronal function to therapeutic early intervention and parental training. The symposium ended with a presentation on the epidemiology of autism; in the context of the data on prevalence, the questions of whether incidence of autism is increasing, and if so, why, were at the forefront of the conference.

The conference, organized by faculty from Hunter College, Weill Cornell Medical College, Memorial Sloan-Kettering Cancer Center, and the Hospital for Special Surgery, originated from discussions between faculty from the Psychology and Biological Sciences Departments at Hunter College. The

---

*aJason B. Dictenberg wrote the first part of this report dealing with the morning session, and Michael J. Lewis covered the second part or afternoon session.
presentations were divided into two sections: one focused on cellular and molecular research, while the other focused on behavioral and therapeutic research. However, the two sections were linked by the recognition that progress in our understanding and ability to alleviate the often devastating impact to the family of the autism spectrum disorders will require parallel progress in both areas.

**Introduction**

Autism spectrum disorders (ASD) comprise a wide range of brain dysfunctions that result in altered social behavior, self-awareness, and language-based interactions. The genetic, biochemical, and cellular biological bases of these brain-altered states are only beginning to be understood. Recent focus in these areas of research has been on connecting genes implicated in autism to their function at the synapse, the structure that ensures the transfer of signals from one brain cell (neuron) to the next. Recent data suggest that genes implicated in autism affect the circuitry of the brain, that is, the wiring that defines the particular geography of synaptic connections between neurons. Hence, genes whose products regulate the structure and function of the synapse are likely to be implicated in neuronal wiring and ASD pathogenesis. The idea that many genes may be implicated independently in autism is supported by recent evidence from cases of inherited autism, which occur secondary to multiple, distinct mutations in neuronal genes required for signaling and synapse regulation. The heterogeneous nature of these genetic abnormalities is consistent with the spectrum of phenotypes and severity of altered behaviors that are observed clinically in autism.

In the early stages of brain development, neurons of the central nervous system (CNS) associate to form billions of connections or synapses. While this process is dynamic and just a fraction of these connections persist into adulthood, the retention of important synapses for brain function involves the fine-tuned expression of genes at precise moments in development. Any cellular or physiological errors that disrupt the proper regulation of synapses may cause brain dysfunction. Current research efforts seek to determine if synaptic dysfunction, as seen in autism and related disorders such as fragile X syndrome, contributes to the mis-wiring of brain circuitry. Efforts to link cognitive dysfunction to circuitry changes in the brain have focused on many levels, including the structure of dendrites that mediates the presynaptic/postsynaptic connection in the majority of excitatory synapses in the brain, the dendritic spine. Pioneering work of Purpura and Marin-Padilla during the 1970s showed changes in the morphology of synapses in the brains of children with mental retardation. These changes were characterized by fewer numbers of synapses (i.e., dendritic spines) and greater spine length. A decrease in the number of spines, and thus the number of synapses, has been found in other disease states involving cognition, such as Down syndrome and Alzheimer’s disease. A change in the morphology of spines seems to be a unifying theme in diseases of cognition, in that the normal pathway of dendritic spine maturation appears disrupted. This results in longer, thinner spines that resemble immature filopodia, or spine precursors. The challenge now is to link these changes in spine architecture and biochemistry to defects in brain organization, function, and ultimately, behavior.

Autism research has expanded to encompass many of the major behavioral and neurobiological research areas. The recognition that the disorder is not just a problem of social or cognitive development has led to the mobilization of research in genetics, biochemistry, and neurophysiology with behavior, cognition, and development to produce a panoply of research wisdom and skills against what we now know is a spectrum of disorders. The application of research findings from developmental psychology in the areas of perception, cognition, and social skills has provided a foundation upon which autism research has progressed. Likewise the recognition by the autism field of learning and memory processes, and the application of strategies that have emanated from them, have provided insight into the etiology and treatment of affected children. The blend of applied behavioral analysis with cognitive neuroscience approaches has been successfully applied to autism with considerable success. Current autism research employs techniques from discrete trial learning, imitation, language comprehension, and perception, among others. The combinations of these with electrophysiological and imaging methods have linked behavioral and cognitive process affected by autism to discrete brain mechanism. There is recognition that the stages of early developmental processes, with the emergence of behavioral and
physiological changes, have provided the timing for such investigations.

In the morning session, five investigators from around the country highlighted their approaches to autism using a range of genetics and diverse brain imaging approaches. The first cohort of researchers at the symposium discussed recent advances that point to activity-dependent genes that function at the synapse, linking the pre- and postsynaptic compartments. Often, these genes are also master regulators of other genes through control of gene expression at the transcriptional and translational level. The products of these activity-dependent genes may be defective in patients with autism since they are highly heritable, and, interestingly, some correlate with language processing function.

In the afternoon session, six other investigators discussed their research. They reported on brain imaging approaches combined with cognitive testing, behavioral intervention strategies, and parent-based support to better diagnose autism earlier and to understand and ameliorate the loss of typical language and joint attention in autism. The symposium concluded with epidemiological research showing possible causes for the increased incidence of autism as characterized today.

**The genetics of autism**

Dan Geschwind, of the UCLA School of Medicine, opened the conference with a discussion of the “autisms” as a spectrum of developmental behavioral abnormalities that results from a spectrum of genetic disruptions (Fig. 1). While many genes have been implicated in the etiology of ASD, and despite the disease being highly heritable (up to 90% penetrant), the mode of inheritance is not well understood. As many as 25% of patients will develop epilepsy at some point in their life, and many also have intellectual disabilities. It appears to affect males more commonly, at a ratio of 4:1 (males to females) and at an overall rate of 1:150–200 births, being more common than any other childhood disease. While many genes have been implicated, it is important to note that no single gene appears to account for more than 5% of autism cases. Therefore Geschwind’s lab focuses on endophenotypes, or biomarkers of behavioral phenotypes that are stably associated with a genetic component.

Geschwind and colleagues discovered that a single gene mutation co-segregated with a language delay in children with autism, and that this mutation encoded polymorphisms in the contactin-associated protein-like 2 (CNTNAP2) gene. CNTNAP2, a member of the neurexin gene family encoding a trans-synaptic protein involved in synaptic formation and maintenance, is expressed in brain regions that are more evolved in primates. Patients with intellectual disabilities, as well as an Amish family with children having focal epilepsy, also carry mutations in this gene; in addition, CNTNAP2 mutations are also highly correlated with...
specific language impairment (SLI), another highly heritable condition that carries no other noticeable developmental abnormalities. Intriguingly, such data connect the \textit{CNTNAP2} gene implicated in a highly specific language learning and memory function with autism; more generally, this connection exemplifies the potential of a gene to impact one component on the spectrum of endophenotypes characteristic of a given disorder (such as autism). Given that language impairment is such a prevalent feature of autism, much work has focused on genes that can account for specific language disorders; other genes, such as those encoding the transcriptional control protein \textit{FOXP2}, are known to cause a monogenic language disorder, although mutations within this gene are rare. This represents another case where gene network interactions highlight a developmentally regulated program to control complex human behaviors. Therefore it is no surprise that the gene encoding \textit{CNTNAP2} functions “downstream” of \textit{FOXP2} regulation in neurons.\textsuperscript{5}

As a member of the neurexin gene family, \textit{CNTNAP2} is one of the best candidates known to link autism with synapses and brain circuits. Neurexins are presynaptic binding partners for postsynaptic proteins termed neuroligins, which have mutated functions in isolated families with multiple autistic family members.\textsuperscript{6,7} These proteins are important for synaptic formation and maintenance, and are implicated in synapse function and proper brain circuit formation. \textit{CNTNAP2} appears to be expressed in highly evolved frontal regions of the human brain and may have played a key role in the evolution of language-based regions in humans (e.g., the anterior perisylvian cortex and the basal ganglia) since this protein is expressed in these areas in non-human primates. Collaborative research between Geschwind’s lab and those of Mirella Dapretto and Susan Bookheimer at UCLA has investigated the proposed role for \textit{CNTNAP2} in autism pathogenesis. This research has shown that risk-allele carriers of \textit{CNTNAP2} do not display a typical response to externally directed task attention. These patients display a stronger local and weaker long-range connectivity in the frontal-striatal circuits than normal subjects using fMRI techniques, lending credence to the hypothesis of disconnections on a circuit level as a basis for part of the behavioral pathology in autism patients.\textsuperscript{8}

Following the genetic heterogeneity of brain diseases of closely related social groups has been of great value in tracing the origin of autism and other related developmental disorders of intellectual disability, including seizure and mental retardation. Of particular significance are studies of rare recessive mutations that are carried within families for many generations, such as within consanguineous marriages. One such study has been pioneered by Christopher Walsh of Harvard Medical School, who discovered a disorder termed microcephaly with seizures (MCSZ). Children with this disease typically exhibit a reduced head size, developmental delay, and mild seizure disorder. By recognizing a similar phenotype in the offspring of consanguineous marriages, such as in certain Middle Eastern populations, they were able to discover the recessive mutation on chromosome 19 that is responsible for this disorder.\textsuperscript{9} While the mutations in the separate populations were distinct, they both affected the \textit{PNKP} gene. How the \textit{PNKP} gene, which encodes for a DNA repair protein, plays a role in the pathogenesis of MCSZ is not yet known. What is clear is that population-specific mutations in a given gene or genes within the same pathway can give rise to a spectrum of phenotypes depending on the effect of the mutations on protein function.

Using a similar approach to discover the genes involved in autism pathogenesis, Walsh and colleagues formed the Homozygosity Mapping Collaborative for Autism, where clinicians and geneticists in the United States and the Middle East study consanguineous families with autism. They found large copy number variation in a section of chromosome 22, an area previously associated with intellectual disability when mutated.\textsuperscript{10} Five regions were deleted on both parental sets of chromosomes, impacting six genes, although none were completely disabled in function. These genes include several of unknown function, ionic gating membrane channel proteins, axonal growth factors, and a transcription factor. Significantly, four of the six genes are expressed in the hippocampus, a region of the brain central to learning and memory function, and an area that is already implicated in dysfunction in autism studies. In addition, some of these genes were previously shown to be activated in response to neuronal activity. This activity-dependent response may be a key mechanism to facilitate the proper regulation of synapse firing during experience-dependent
changes in synaptic strength (the cellular correlate of learning and memory). A dysregulation of any of these genes could have direct implications for altered synapse formation and function during brain development.5,11

Further probing the nature of the deletions and the effect on these genes led Walsh and colleagues to use microarray technology, focusing on a single copy number variant that deleted a region near the RNF8 gene, a transcriptional co-regulator.10 Transcription factors including RNF8 are proteins that can rapidly switch genes on or off in response to cellular signaling and growth cues. The deletion affected the transcription site of the RNF8 gene in a region that contained a CREB binding site. CREB is another major transcription factor that is mobilized in response to activity to turn on genes that support long-term changes in synaptic strength. Removal of this binding site for CREB will likely have significant effects on RNF8 gene expression and subsequently on the genes that require this cofactor for their expression. Future studies will determine which genes these are and in which direction they are dysregulated in response to the RNF8 mutation.

Taken together, these genetic studies reveal the heterogeneity of causes of autism that parallel the spectrum of observed phenotypes of human behaviors among carriers of these mutations. While the nature of these mutations is only beginning to be revealed, family pedigrees of recessive mutations have implicated genes central to neuronal function, such as those functioning at synapses. A paradigm example of this is the CNTNAP2 gene encoding a trans-synaptic protein mediating synapse formation and function, and its proposed partner neurexrin, which binds to CNTNAP2 family members to bridge pre- and postsynaptic compartments. Loss of function of these genes has direct implications for loss of proper circuit formation, and may lead to synaptic dysconnection during development.12 In addition, it appears that seizure disorders such as epilepsy are common to several distinct brain pathologies that involve developmental delay, and therefore this excessive activity may contribute to defects in learning and behavior that manifest in ASD.

**Gene expression, synapses, and circuit formation**

Coming from the other end of the molecular spectrum, Jason Dictenberg of Hunter College is pioneering work on the monogenic causes of autism at the molecular level of the synapse. Fragile X syndrome (FXS) is the leading single-gene cause of autism, presently estimated at ~3–5%. Strikingly ~60% of children with FXS have autistic behavior.13 The fragile X protein FMRP, an mRNA-binding protein, is a master regulator of mRNA transport to dendrites. It is also involved in the translation of synaptic genes in response to neuronal activity.14 FXS is caused by a trinucleotide(CGG)-repeat expansion in the 5′ untranslated region of the FMRP gene that leads to transcriptional silencing secondary to hypermethylation. The mutation is highly heritable and appears to be exaggerated upon generational inheritance. Dictenberg and colleagues use a mouse model of FXS to study synaptic defects in genes implicated in circuit formation and function.

A hallmark of synaptic changes in FXS is the preponderance of long, thin spines that are reminiscent of immature stages during development. Dictenberg and colleagues used a novel RNA-tagging method to demonstrate the role of FMRP in mRNA transport to dendrites in response to neuronal activity. They found that loss of FMRP led to decreased mRNA targeting to dendrites and altered spine morphology.15 One example of a specific mRNA affected is the CaMKII-alpha mRNA, which is transported to dendrites in response to glutamatergic signaling to facilitate learning and memory. However, experiments carried out in the mouse model of FXS demonstrated that the processivity (run length) of individual CaMKII-alpha mRNA particles was diminished following metabotropic glutamate receptor (mGluR) signaling. Previous work has shown that, in the absence of FMRP, hippocampal CA1 neurons undergo excessive long-term depression of synaptic responses upon mGluR signaling in the mouse model, a process that normally requires local protein synthesis within dendrites.16 Therefore loss of mRNA transport and subsequent dysregulation of local protein synthesis may contribute to defects in synaptic plasticity, learning, and memory function observed in FXS. Dictenberg hypothesizes that a loss of stimulus-induced mRNA transport may lead to precocious mRNA translation before the mRNAs reach the dendrite, with proteins therefore made at the wrong place and time. This mechanism may be common to many of the heterogeneous causes of autism.
Recent data from the Dictenberg lab explores the role of trans-synaptic cell adhesion molecules and their potential dysregulation in FXS. Neureligins (NLs) are postsynaptic proteins that are implicated in autism pathogenesis in cases of familial inheritance. The proteins are binding partners for the CNTNAP2 family of presynaptic neurexins.\(^6\),\(^7\),\(^17\)

Importantly, Dictenberg made the connection that one of the known mRNA targets of FMRP, PSD-95, is a scaffold protein that can regulate the levels of NLs at synapses.\(^18\) Therefore, dysregulation of PSD-95 expression, as seen in neurons from the mouse model of autism, implicates NL dysfunction. The lab uses super-resolution microscopy methods to quantify changes in proteins at individual synapses upon neuronal activation (Fig. 2). The data show that mGluR stimulation causes a rapid increase (within 15 minutes) in dendritic PSD-95 protein levels in wild-type hippocampal neurons, but that this up-regulation is absent in neurons derived from the FXS mouse model of autism. NL1 protein also appears altered in its expression in FXS neurons, and this altered the ratio of excitatory to inhibitory synapses. Live cell imaging of PSD-95-GFP and NL1-GFP fusion proteins expressed in hippocampal neurons shows dynamic movements within filopodia and spines. Analysis of these protein movements in neurons from FXS mice will lead to a greater understanding of the defects that may give rise to altered plasticity and synapse structure. Future work from the Dictenberg lab aims to determine how an alteration of the balance of excitatory and inhibitory inputs in diverse brain regions contributes to the observed physiological and behavioral changes in FXS and autism, and how excessive activity such as in epilepsy may contribute to synaptic defects and altered circuit architecture.

Looking into the visual system for clues on circuit formation and function, Hollis Cline and her group at Scripps Research Institute turned to the tadpole eye and the innervation pattern of the optic tectum. They used fluorescent proteins

---

**Figure 2.** Cultured hippocampal neuron (21 days *in vitro*) from mouse brain showing how super-resolution microscopy can highlight individual synapses. Here neurons are stained using immunofluorescence with antibodies to synapsin (red), microtubule-associated protein 2 (MAP2, blue), and the actin cytoskeleton (green). Note the colocalization of actin-rich dendritic spines (green) with the presynaptic axonal boutons (red) along the length of the dendrites (blue) where hundreds of synapses can be visualized. Courtesy Jason Dictenberg, Hunter College.
expressed in these animals and, owing to the relative transparency of these animals, were able to visualize the axonal projections and their changes in connectivity over time. The projections were modified by visual experience as predicted by early experiments by LeVay, Hubel, and Wiesel. These pioneering scientists showed that sensory experience shaped the developing visual cortex during a critical period in brain development and that sensory deprivation led to loss of connectivity in that represented region. Cline used this approach to look into dendritic arborization in response to synaptic activity, a process that is less understood than axonal innervation. Her group showed a dramatic rearrangement of dendritic arbors in less than an hour and, over longer periods, demonstrated an extensive elaboration with almost every process undergoing some change. Through careful analysis this work showed an iterative process whereby branches were elaborated and then subsequently pruned to allow stabilization of only a select few processes until the mature circuit formed. This was consistent with the synaptotrophic hypothesis, whereby synaptic connections control the exploratory behavior of developing neurons, the development of neuronal arbors, and the establishment of neuronal circuits. Visual stimulation was shown to enhance this arborization, and electrophysiological experiments showed the strengthening of existing synapses and the addition of new synapses as well, further confirming the validity of the hypothesis.

Cline’s lab observed not only these changes in neuronal excitability and arborization upon sensory stimulation, but also modification of gene expression. In searching for an activity-dependent mechanistic cause to explain these broad-ranging effects of visual stimulation on the brain, her group looked to mRNA-binding proteins like FMRP that control mRNA stability, transport, and translation. They focused on CPEB, which binds to cytoplasmic polyadenylated mRNA tails to control their subsequent translation upon neuronal activity. Using a dominant-negative form of the CPEB protein that interferes with normal CPEB function, they looked to the effects on arborization in the tadpole visual circuit. The mutant protein diminished the overall growth rate of the dendrites and the branch length, which dampened the circuit formation.

Together, the studies of Cline and Dictenberg highlight the role of gene expression in controlling synapse formation, structure and function, and how synaptic activity can alter gene expression to modify synaptic inputs and the topography of the overall circuit. Cline’s elegant work highlights the plasticity of the circuit with regard to sensory input and dendritic morphology, and shows how this can affect the brain on a macroscopic level over longer periods of time.

The connectome

Bringing the theory of genomics to the study of synaptic connections, Jeffrey Lichtman of Harvard University discussed the architecture of the wiring of human brains and suggested that most diseases of the nervous system could be a result of aberrant effects on the circuitry of neurons. The work of his lab aims to build on the observations of the early neuroscientists Golgi and Ramon y Cajal, who discovered the interconnectivity of the neurons in the brain and speculated on the directionality of information flow from one neuron to the next within a circuit. The wiring of individual neurons within the human brain is astoundingly complex. Therefore Lichtman’s lab uses advances in imaging technologies to better assess connectivity. Similar to the way that geneticists have mapped out the genome, his group seeks to map the human “connectome” to have a working diagram of an apparently normally wired brain. He acknowledged that “normally wired” may be inaccurate, since genes are expressed uniquely within individuals despite sharing over 99% genetic similarity. These individual expression patterns may affect neuronal connections so that each person has refined connections that are uniquely based on experience-dependent learning and plasticity. Despite these limitations, Lichtman’s group has advanced the connectome project by applying computer-assisted image acquisition and analysis to the structural mapping of sets of neuronal circuits and the nervous system as a whole. Once the connectome is mapped, withstanding some accepted diversity in the wiring, this knowledge could be applied to animal models of neurological diseases, like autism, to determine the defects in wiring architecture that may give rise to altered learning, memory, and behavior.

Lichtman’s group has shown differences in the developmental connectivity of mouse brain using the neuromuscular junction (NMJ) as a model system. The NMJ is where the motor nerves that originate in
the spinal cord innervate muscle peripherally. Early after birth there exist multiple neurons (axons) that contact the same muscle fiber junction. After a few weeks in mice (and months in humans), a pruning of these connections takes place such that eventually only a single axon innervates the junction. The other axons retract and find other targets, resulting in a smaller motor unit in the fiber that is stable throughout the lifetime of the animal. This process of multiple innervation followed by pruning during development occurs in the central nervous system as well, and the mechanisms for this process appear to be similar.

To better follow the individual connections of axons with the muscle fibers during development, the Lichtman lab used spectrally distinct fluorescent protein markers to visualize individual fibers. Using time-lapse imaging methods, they observed in vivo the initial innervation and synaptic competition, subsequent synaptic formation, and finally synapse retraction during development of the mouse. Expanding on this approach, they created a new line of transgenic mouse that expresses three different spectral varieties of fluorescent proteins within the brain. By driving the expression of each color (blue, green, and red) randomly within each cell, they were able to create a “brainbow” mouse where each cell has a distinct color variation from the next. Given the complexity and density of packing of individual fibers within the central nervous system, this technique serves to disambiguate each neuronal process even within the context of hundreds or thousands of neighboring processes (Fig. 3). Using these mice, Lichtman discovered that an axonal connection at the NMJ that is dominant, or one that occupies most of the junctional space, may not always win out over the lesser-connected axons. The neuron of the “winning” axon, however, was always observed to be successful at adjacent synapses where another branch of its axon innervated a distinct NMJ. These results suggest that the “successful” neuron is able to express synaptic molecules that enable it to strengthen its synapses more efficiently than other competing neurons in response to activity at the NMJ.

Applying new technological advances in tissue sectioning along with the fluorescent labeling technique, Lichtman’s group was able to demonstrate the highly individualized nature of axonal innervation by mapping the connectome of the interscutularis muscle of the mouse ear. Using a serial section technique that preserves a continuous connection between subsequent slices, they generated a strip of tissue that spans the whole muscle, leaving its neuronal processes intact. Upon examination of the

---

**Figure 3.** Connectomics with no tracing necessary! Brainbow mouse sections of the peripheral nervous system neuromuscular junction (NMJ) highlight the use of randomized fluorescent protein expression within each neuron. Individual axons can be distinguished from each other as they enter the muscle (left) and at the synapse (right) using this technique as they innervate the NMJ during development. Slide courtesy of Jeff Lichtman.
connectivity, they found that, strikingly, between genetically identical animals, the patterns were distinct (i.e., like the distinct fingerprints of identical twins). This individuality included arbor shapes, branching patterns, and the number of branches. Also noticed was a suboptimal pattern of connectivity, with branches often passing a connection before looping back to make a synapse, which Lichtman says is evidence that the connectome is to some degree built “on the fly” without pre-programming. He believes that the patterning of synapse elimination “unfetters the mammalian nervous system from the tyranny of the genes.” In the end, each animal ends up with a very different nervous system, largely due to environmental influences and experiences, through basic learning and memory mechanisms. Ultimately, connectomics research may allow investigators to compare brains of autistic patients to normal individuals to determine which circuits are disrupted and how this correlates with behavioral differences.

**New directions in early detection and intervention in autism**

The second keynote address was presented by Geraldine Dawson (Fig. 4), chief science officer of Autism Speaks, an autism research and advocacy organization. She provided an overview of the research on early diagnosis of ASD and clearly articulated the consensus in the field that there is a need to accelerate the identification and treatment of ASD. She pointed out that while there have been deficiencies of the health care system in recognizing the need for early diagnosis, there are now several early childhood screening options, including some that appear to be useful in identifying signs of autism as early as six months. In agreement with the speakers of the morning session, Dawson indicated the importance of the genetic components of ASD and the fact that siblings of autistic children are at much higher risk for the disorder. This provides an opportunity for prospective research on these infants that may assist with the detection of signs of autism even earlier.

Dawson and others have shown that up to six months of age, children who will go on to develop autism typically seem fairly normal. Deficits in attention and selective engagement with human faces and voices begin to appear at this age and increase in severity to one year when the social and communicative deficits of autism can clearly be identified.
in most ASD children. It is important to note, however, that between a quarter and a third of children who develop ASD do not show deficits until one or two years of age.

Dawson described innovative research that shows promise for recognizing the early signs of autism. First, she discussed research indicating that high-risk infants have diminished ability to distinguish lightness and darkness in the visual field and that this deficit may play a role in the inability to respond to human faces. Next, Dawson presented groundbreaking research identifying deficits in event-related potentials (ERPs) to human faces in a specific cortical region, the fusiform gyrus, of high-risk infants. The deficit is characterized as diminished amplitude in this electrophysiological pattern that is not lateralized to the right hemisphere as it is in low-risk infants. She states that the challenge is to determine whether these and other promising findings are predictive of the disease or whether they are endophenotypes indicating a predisposition to the disease.

Early intervention has been shown to have an impact on learning and language development of ASD. Dawson asserts that along with the successful strategies from applied behavior analysis and discrete trials training, the use of methods from developmental psychology that take advantage of the “intuitive” learning of children as they actively explore their environment might produce the most effective intervention strategy. This thinking emphasizes comprehensive approaches to intervention and is at the heart of Dawson’s collaboration with Sally Rogers in the Early Start Denver Model (ESDM), a comprehensive intervention program for young children with autism. The ESDM Program is a collaborative effort of experts—physicians, psychologists, behavioral therapists, speech pathologists, and occupational therapists—who work to intervene on as many ASD deficits as possible. Social development with language, motor, and cognitive skills are intensively applied to autistic children. She states, “We’re building a baby from the ground up in all senses of the word.” Their promising findings using this approach have encouraged Dawson, Rogers, and their team to expand the program to other sites. They believe that programs that bring together therapists, experts, and parents in early intervention offer the greatest promise of improvement for those with ASD.

### Social motivation, attention, and learning in the autistic brain

Early in development, infants preferentially focus their attention on human faces and voices—particularly the face and voice of their mother—while infants with ASD do not show this preference. Dawson discussed how the ERP response to faces is diminished in autistic children. The fusiform “face area” and the amygdala, a key brain region involved with emotion, exhibit reduced amplitude ERP responses in children with ASD.

Mirella Dapretto, at the University of California, Los Angeles, and colleagues have investigated these findings from the perspective that autism may involve the alteration of brain function in areas that mediate “theory of mind,” or the interpretation of others’ intentions, actions, and emotional states. Her research has shown that although deficits in the brain regions associated with theory of mind are observed when unfamiliar faces are presented to autistic children, the response is normative when familiar faces are presented. The deficit appears to be associated with attentional mechanisms: when autistic children are instructed to pay attention during testing, they exhibit normal levels of activity in these brain areas.

With this evidence that brain function is essentially intact in these areas, Dapretto shifted her focus to explore the lack of preference for facial and vocal stimuli in ASD. “According to the social motivation hypothesis, this lack of an attentional preference for these stimuli may reflect that they’re not ‘rewarding’,” she said. These stimuli typically produce reinforcement in unaffected children but not in children with ASD. Her research showed that social and nonsocial reinforcement that activates the brain reward regions, such as the ventral striatum, fails to do so in children with ASD. The difference was particularly striking with social rewards.

These deficits suggest that brain centers that mediate emotion, especially emotion generated in social situations, may be impaired. Dapretto and colleagues have performed experiments in which ASD children were instructed to mimic facial expressions that show different emotions. Although the children performed well on the task behaviorally, functional magnetic resonance imaging
Lewis & Dictenberg Autism meeting report

Figure 5. Mirror neuron system (MNS) activity in ASD children during imitation. The effect of imitation of emotional expressions on a MNS. Event-related fMRI images of typically developing children (TD) and autism spectrum disorder (ASD) children and the differential comparisons of these children (TD vs. ASD). Higher activity is found in the right pars opercularis of the inferior frontal gyrus in TD children but not in the ASD children. The between-group comparison shows the significant effects. Differences in the MNS are believed to underlie the deficits in social and emotional development of ASD children. Lower image: $P < 0.05$, corrected at cluster level.

(fMRI) shows that the children exhibited deficits in important brain regions in comparison to unaffected children (Fig. 5). In particular, they showed lower levels of activity in brain areas with “mirror neurons.” These are regions of the brain that are thought to be necessary for following and interpreting movements and intentions of others, and may be key to understanding social and emotional situations. Dapretto’s research is consistent with others’ in suggesting that this system may be impaired in autistic children. Dapretto indicated that there may be a link between the deficits in mirror neuron activity and attentional bias seen with autism; recent data from her lab indicates that there is a lack of coordination between the reward system and mirror neuron centers with ASD. According to Dapretto, children who have highest activity in the ventral striatum (when they were getting the smiling faces as positive feedback) were also the children who showed greater activity in those mirroring regions that are thought to be important for interpreting another’s facial expression and feelings. Dapretto concluded by stating that the social and emotional impairments observed with ASD may be due to deficits in the mechanisms that mediate long-range integration between functioning brain regions. This could be exacerbated by impaired attentional bias. Her current research is focused on the role these differences may play in the deficits observed with the development of linguistic abilities.

Language in ASD: from behavioral phenotypes to neurobiology and genetics

Language deficits are a common feature of ASD. Exploring the range of these deficits is the focus of work by Helen Tager-Flusberg (Boston University) and her colleagues. They have examined the differences of those who have intact language skills (autism language normal; ALN), who may still have diminished abilities to understand social cues and the embedded meaning of speech (often labeled Asperger’s syndrome) compared with those with more severe language deficits (autism language impaired; ALI). ALI children were found to exhibit deficits that are surprisingly similar to those diagnosed with SLI on language tests. Tager-Flusberg and colleagues showed that ALN did not show these deficits. With these findings as a clue, she and her colleagues explored the brain regions that mediate language to determine if the deficits produced identifiable anomalies. She found that ALI children show impairments in the left hemisphere lateralization of speech. Typically, the development of language is associated with increased left hemisphere dominance of Broca’s area in the frontal cortex (the speech production area) and Wernicke’s area in the temporal cortex (the speech recognition area). Autistic children exhibit little left hemisphere dominance or even reversed dominance of Broca’s area concomitant with an exaggerated left
dominance in Wernicke’s area. Tager-Flusberg and colleagues found that this profile was associated with ALI children, and not ALN children. She and her colleagues investigated other language regions, exploring the arcuate fasciculus, the major pathway connecting Broca’s and Wernicke’s areas. Using diffusion tensor imaging of the arcuate fasciculus, they found no apparent differences between ADS children and unaffected controls. However, when ASD children were divided on the basis of linguistic ability, it was apparent that ALI showed diminished connectivity between the two speech areas.

Siblings of autistic children are at greater risk for developing autism, and they exhibit reduced language performance and similar alterations of brain language centers as their affected siblings. This was exemplified by Tager-Flusberg and colleagues’ work in which they found that siblings showed lower language and reading performance and similar anomalies of Broca’s and Wernicke’s areas as ASD children. Since not all of these siblings will go on to have ASD, it suggests that the language impairments and altered brain systems that mediate language represent an endophenotype for ASD.

Tager-Flusberg also reported preliminary data on identification of basic speech sounds at ages six months and nine months as they relate to autism. With normal development, there is a change in recognition of these sounds associated with greater brain lateralization at the later stage of development. Infants at risk for ASD exhibited no change in recognition and a lack of asymmetry in brain development at age nine months. These data suggest that there may be a seven- to nine-month period during which developmental abnormalities can be detected and followed as indicators of risk for the development of ASD. Identification of genetic markers that are associated with the language deficits during this period may lead to markers useful in identifying risk for ASD. A finding such as that reported by Geschwind earlier in the symposium indicating a mutation in the CNTNAP2 gene that is passed on maternally and is associated with language development is very encouraging and is being pursued by Tager-Flusberg and colleagues.

Integrating neuropsychology, development, behavior, and treatment

Sally Rogers, at the University of California, Davis, described how we have come to understand the essential features of autism. We now understand that ASD is manifested differently in early childhood in comparison to school age. She described how the various cognitive impairments of affected two to five year olds can be grouped in “clusters” of social, emotional, action-oriented, linguistic, perceptual, and learning deficits.

In the 1990s, autism research emphasized a model in which the symptoms of autism were seen as a function of the impact of biological and environmental factors on cognitive processes. Therefore, treatment strategies tended to selectively target the problem areas with the hope that there would be improvements in symptoms associated with them. Along these lines, strategies that teach imitation to ASD children have been shown to improve symbolic play, joint attention, and language skills.

More recently, an alternative model has gained favor: one that ties the symptoms of ASD to dysfunctions in the structure and connectivity of specific brain regions. Rogers reflected on Geschwind’s earlier presentation as an example of this approach. There is a growing body of information that ASD is associated with a diminished capacity for the formation of long-range circuits that are essential for the integration of complex brain functions. She believes that these circuits are essential for the development of complex skills that are deficient in ASD. In addition, she described a different potential problem associated with the development of these critical circuits. As discussed in Lichtman’s presentation in the morning session, there may be deficits in the formation of brain circuits associated with a lack of “pruning” of local brain circuits that result in over-connectedness within these systems, thereby leading to disorganization at the local level. Rogers believes that interventions that include teaching imitation behavior provide the stimulation for the development of efficient circuits. There is evidence that when engaging in these tasks, ASD children (even the high functioning individuals) show diminished and more localized brain activity than their unaffected peers. She believes that early learning of these and other skills is the key to producing the essential neuronal circuits. Early interventions can bolster the development of neuronal circuits that are probably less robust in those with ASD. Although time consuming, the repeated exercises of discrete trial training and other behavioral methods of applied
behavioral analysis are consistent with this brain-oriented approach.

A series of other approaches, particularly those that include naturalistic teaching, show great promise. Allowing children to determine the choice and focus of activities in the context of daily programs with adults leads to the development of important skills and is similar to what parents do with their children. In this context, Rogers explored her research with Dawson on the ESDM.31 Using a video presentation of an ESDM training session, she illustrated the range of situations and reinforcements employed by the program. Social interactions, including eye contact, motor activity, and affirming emotional feedback, provide effective reinforcement that is evident. The focus is primarily on the various neuropsychological deficits of the early stages of ASD. The hope is that active play in the context of the developmental training program will lead to the acquisition of skills that then permits cognitive and social advancement, which may in turn allow them to catch up to their peers. She presented additional data from a University of Washington study described by Dawson showing that the percentage of children that met diagnostic criteria for intellectual disability in the sample decreased from 70% to 30% after two years of the program. She concludes that these findings are evidence of the potency of early learning and experiences in the acquisition of cognitive, social, and motor skills. Early intervention with ESDM and similar programs that emphasize an intensive “integrative” approach will prove invaluable in the development of the essential neural networks in young ASD children and lead to better treatment.

A parent-mediated intervention

The establishment of joint attentional focus between infant and adult is essential for the development of early language acquisition. Michael Siller at Hunter College views early language learning as a collaborative process in which a child and adult both attend to the same object or task in parallel. The basic processes associated with this state are quite powerful and the failure of their development may be a major factor in the language deficits in autism. Siller’s research suggests that the deficits in joint attention, which are common in ASD children, make it exceedingly difficult for children to effectively pair words with their meanings. Typically, children between 15 and 19 months are able to recognize the focus of another person’s attention, and as a result label the object. Before this age, language as well as other learning depends on the parental establishment of joint attention. “What you hear a lot is parents’ labeling objects to which the child is already attending, or commenting on actions or intentions or goals that the child is pursuing,” he said.

Siller, in collaboration with Marian Sigman at UCLA, has shown that a child’s capacity for joint attention correlates directly with their subsequent acquisition of language skills.39 Siller and Sigman described how joint attention deficits are observed in children with ASD, probably disrupting language development. Longitudinal studies by Siller and Sigman also suggested that autistic children benefit from a process sometimes used by mothers with their autistic children, a process called maternal “synchronization.” With synchronization, the mother continually uses engaging language to talk about and describe the objects and actions of a child’s attention, thereby mitigating their language deficits.

Recently Siller and colleagues have investigated the processes that are the basis of the benefits of synchronization and have been developing an intervention program to train parents of ASD children in the effective use of responsive language with their children. Data from his recently completed randomized controlled trial indicate that an intervention designed to effectively train individuals to use these techniques could be implemented and prove effective in stimulating language development in autistic children. The program involves 12 in-home training sessions in which both parent–child and interventionist–child interactions are employed. Conventional teaching, live modeling, and coaching methods are used in the program. Each session is recorded on video and subsequently reviewed, enabling parents and interventionists to review progress and make adjustments to improve the success of the intervention.

Siller reported on a pilot study to aid in the development of the study guide for the intervention involving a small sample. His team partnered with the California Regional Centers to recruit a larger sample of families from the Los Angeles metropolitan area for a randomized controlled trial. Children in the trial had a clinical diagnosis of ASD, severe language impairment, and were six years of age or younger. The group using the training showed improvements in the use of maternal synchronization over their performance at the beginning.
of the study, thus indicating that the intervention procedures were effective in training parents in the use of responsive language. Further examination of the data is required to determine if there were long-term gains in language resulting from this enhanced synchronization. Siller and colleagues are currently analyzing the first year follow-up data. He is also collaborating with Connie Kasari at UCLA to explore the possibility of a similar intervention with younger children (18 to 30 months old).

**Epidemiology and the changing paradigm of ASD**

The concluding presentation given by Marshalyn Yeeargin-Allsopp provided a review of ongoing efforts of the CDC to determine the prevalence of ASD in the United States and provide information on the factors that contribute to changes in these statistics (Fig. 6). She began by listing some of the factors that have confounded previous efforts to accurately measure the incidence of autism. Most of these confounds are related to the quality of the measurement criteria, including quantification of onset based on diagnosis at a relatively late age (averaging 4 to 6 years old), failure to confirm diagnoses over the course of a study, and the use of inaccurate or outdated diagnostic standards.

This latter factor is a crucial consideration in current attempts to measure prevalence. Yeearkin-Allsopp provided a brief update on the change in criteria. This change is especially important considering that autism was first described in 1956 by Leo Kanner and that in 1980 this disorder was reclassified as a developmental disability rather than a mental illness. Other milestones are the inclusion of ASD in the World Health Organization’s ICD-10 in the 1990s and in the American Psychiatric Association’s DSM-IV, both of which considerably broadened the description of ASDs and included high-functioning individuals such as those with Asperger’s syndrome. She indicated that an increase in prevalence has occurred in parallel with the use of the broader, more nuanced diagnostic criteria. This has made it difficult to understand the changes in prevalence over time, which have been dramatic: increasing from 4–5 in 10,000 individuals prior to 1990s standards to 6 cases per 1,000 individuals more recently. Yeeargin-Allsopp stated that prevalence trends are limited by the changes in the characterization of the illness, frequently leading to contradictory interpretations.

Yeeargin-Allsopp’s team at the CDC uses a multiple source record review in their efforts to accurately determine trends. Using this strategy to monitor ASD along with other developmental disorders, they found that the prevalence among eight-year-olds in the five counties of metropolitan Atlanta showed a prevalence of 6.5 per 1000 in the year 2000. These data were surprising because its peak prevalence exceeded all disorders being studied, including diseases such as cerebral palsy, hearing loss, or visual impairment.

In light of such statistics, the U.S. Congress expanded the funding of this program and the Autism and Developmental Disabilities Monitoring (ADDM) Network to now encompass multiple sites across the nation. Surveillance at all locations

![Figure 6. Summary and historical perspective on autism prevalence before 2009, as assessed by the CDC (Yeeargin-Allsopp).](image-url)
incorporates records from multiple health and education sources, with all cases subject to review and confirmation by clinicians using DSM-IV criteria. “Our results have really become the standard for setting ASD prevalence estimates for the United States,” said Yeargin-Allsopp.

Surveillance data from 14 different sites for the year 2002 monitored nearly 10% of American children for ASD. The average prevalence was 6.6 per 1,000—a measurement that is the basis for the 1:150 statistic that was cited throughout the symposium. The conclusion, based on this rate of prevalence, is that approximately 560,000 Americans under the age of 21 are affected by the disorder. In December 2010, the CDC reported on data collected from 11 sites in 2006 that indicated an even-higher prevalence of 1 in 110 children, a 57% increase since 2002. As with previous surveys, the disparity between boys and girls remains approximately the same—boys are more than four times more likely to be diagnosed with ASD than girls. These data are consistent with those of Europe and Asia. The current criteria for development of ASD include onset by the age of three; however, the average age of diagnosis of the disorder is considerably later at four and one-half years of age. In agreement with many of the other presentations, Yeargin-Allsopp stated, “We obviously still have a lot of work to do in terms of identifying children with these behaviors early.”

Conclusion

Having Marshalyn Yeargin-Allsopp of the CDC conclude the symposium put ASD in the context of the national debate. The CDC data and other sources around the world suggest an epidemic of ASD among various demographic groups across the United States. In the past few decades, the number of children with ASD has gone from around 4 cases per 10,000 children to as high as 1 in 150 in some areas. Although the precise reasons for this precipitous rise are unclear, previous efforts to accurately measure autism were flawed, and the definition of those included on the spectrum has recently broadened considerably. She concluded that fully exploring and addressing this problem will require a concerted effort among scientists, clinicians, and governmental and non-governmental agencies. New detection methods now show potential to identify high-risk infants as early as 6 months of age. The detection of changes in language recognition with development in typical children was notably absent in autistic children, and a novel paradigm of maternal “synchronization” with an ASD child’s attention was shown to be effective in stimulating language development. A new understanding of the genetic abnormalities that may contribute to the cause of autism was highlighted, with both large mutations (such as with copy number variations that delete the RNF8 gene) and single gene mutations (such as with the fragile X gene and the contactin-associated protein, CNTNAP2) playing a significant role in the etiology of ASD. In addition, the function of these genes in the formation of circuits, which are being mapped in the human brain currently, is an exciting area of research that is just beginning to open up new possibilities for treatment. The fact that epilepsy is a highly comorbid factor with ASD suggests that excessive excitatory activity during brain development may contribute to the defects in learning, behavior, and brain wiring observed in ASD; this must be addressed in future studies using anticonvulsant drugs to determine which types of ASD may respond favorably. The presenters and attendees of the symposium benefited from the knowledge exchange at the intersection of cell biology, neuroscience, developmental psychology, and public health on the understanding and treatment of autism. Sharing the results in these disparate fields should fuel new and exciting research that may one day alleviate the pain and suffering experienced by patients and families affected by autism.

Acknowledgments

This publication was made possible by Grant Number RR003037 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH).

References


